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A study of selected antineoplastic, antibiotic, and corticosteroid drugs in intravenous admixtures : a thesis ...

Melvin Philip McRae
University of the Pacific

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A STUDY OF SELECTED ANTINEOPLASTIC,
ANTIBIOTIC, AND CORTICOSTEROID
DRUGS IN INTRAVENOUS ADMIXTURES

A Thesis
Presented to
the Faculty of the Graduate School
the University of the Pacific

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
M. Philip McRae
July 1972

This thesis, written and submitted by

Melvin Philip McRae

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Dated July 31, 1972

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M. P. M.

University of the Pacific
Stockton, California
July 1972

TABLE OF CONTENTS

	Page
LIST OF TABLES	iv
LIST OF GRAPHS	v
LIST OF FIGURES	vi

Chapter	
I. INTRODUCTION	1
Problems of Intravenous Admixtures	3
Methods of Predicting Intravenous	
Incompatibilities	22
Recent Studies of Intravenous Admixtures	24
Oncolytic Drug Admixtures in Intravenous	
Chemotherapy	28
II. EXPERIMENTAL PROCEDURE	36
Preliminary Study	37
Analyses of Admixtures	38
III. RESULTS	42
IV. DISCUSSION	110
V. SUMMARY AND CONCLUSION	121
BIBLIOGRAPHY	123

LIST OF TABLES

Table	Page
I. Frequency of Additives Included in Intravenous Solutions (Percentage Breakdown Includes Only Those I.V.'s Which Contained Additives)	13
II. Frequency of Additives Included in Intravenous Solutions (Percentage Breakdown Includes Number of Additives in All I.V.'s Surveyed)	14
III. Summary of Analyses of Cytotoxic Admixtures Using Ultraviolet Spectrophotometric Examination	108
IV. Change in pH When 5-Fluorouracil Was Added to Methotrexate Sodium in 5 percent Dextrose Injection	116

LIST OF GRAPHS

Graph	Page
1. Beer Plot for Prednisolone Sodium Phosphate (λ_{max} 257 m μ) in 5 percent Dextrose Injection	43
2. Beer Plot for 5-Fluorouracil (λ_{max} 265.5 m μ) in 5 percent Dextrose Injection	44
3. Beer Plot for Methotrexate Sodium (λ_{max} 302 m μ) in 5 percent Dextrose Injection	45
4. Beer Plot for Cytarabine (λ_{max} 272 m μ) in 5 percent Dextrose Injection	46
5. Beer Plot for Sodium Cephalothin (λ_{max} 236 m μ) in 5 percent Dextrose Injection	47
6. Beer Plot for Vincristine Sulfate (λ_{max} 256.5 m μ) in 5 percent Dextrose Injection	48

LIST OF FIGURES

Figure		Page
1.	U.V. Spectrum of 5-Fluorouracil (λ_{\max} 265.5 m μ) with Prednisolone Sodium Phosphate (λ_{\max} 257 m μ) in 5 percent Dextrose Injection at one hour	51
2.	U.V. Spectrum of 5-Fluorouracil (λ_{\max} 265.5 m μ) with Prednisolone Sodium Phosphate (λ_{\max} 257 m μ) in 5 percent Dextrose Injection at four hours	52
3.	U.V. Spectrum of 5-Fluorouracil (λ_{\max} 265.5 m μ) with Prednisolone Sodium Phosphate (λ_{\max} 257 m μ) in 5 percent Dextrose Injection at eight hours	53
4.	U.V. Spectrum of Methotrexate Sodium (λ_{\max} 302 m μ) with 5-Fluorouracil (λ_{\max} 265.5 m μ) in 5 percent Dextrose Injection at one hour	56
5.	U.V. Spectrum of Methotrexate Sodium (λ_{\max} 302 m μ) with 5-Fluorouracil (λ_{\max} 265.5 m μ) in 5 percent Dextrose Injection at four hours	57
6.	U.V. Spectrum of Methotrexate Sodium (λ_{\max} 302 m μ) with 5-Fluorouracil (λ_{\max} 265.5 m μ) in 5 percent Dextrose Injection at eight hours	58
7.	U.V. Spectrum of Cytarabine (λ_{\max} 272 m μ) with 5-Fluorouracil (λ_{\max} 265.5 m μ) in 5 percent Dextrose Injection at one hour	61
8.	U.V. Spectrum of Cytarabine (λ_{\max} 272 m μ) with 5-Fluorouracil (λ_{\max} 265.5 m μ) in 5 percent Dextrose Injection at four hours	62
9.	U.V. Spectrum of Cytarabine (λ_{\max} 272 m μ) with 5-Fluorouracil (λ_{\max} 265.5 m μ) in 5 percent Dextrose Injection at eight hours	63
10.	U.V. Spectrum of Prednisolone Sodium Phosphate (λ_{\max} 257 m μ) with Cytarabine (λ_{\max} 272 m μ) in 5 percent Dextrose Injection at one hour	65

Figure		Page
11.	U.V. Spectrum of Prednisolone Sodium Phosphate (λ_{\max} 257 m μ) with Cytarabine (λ_{\max} 272 m μ) in 5 percent Dextrose Injection at four hours	66
12.	U.V. Spectrum of Prednisolone Sodium Phosphate (λ_{\max} 257 m μ) with Cytarabine (λ_{\max} 272 m μ) in 5 percent Dextrose Injection at eight hours	67
13.	U.V. Spectrum of Sodium Cephalothin (λ_{\max} 236 m μ) with 5-Fluorouracil (λ_{\max} 265.5 m μ) in 5 percent Dextrose Injection at one hour	70
14.	U.V. Spectrum of Sodium Cephalothin (λ_{\max} 236 m μ) with 5-Fluorouracil (λ_{\max} 265.5 m μ) in 5 percent Dextrose Injection at four hours	71
15.	U.V. Spectrum of Sodium Cephalothin (λ_{\max} 236 m μ) with 5-Fluorouracil (λ_{\max} 265.5 m μ) in 5 percent Dextrose Injection at eight hours	72
16.	U.V. Spectrum of Methotrexate Sodium (λ_{\max} 302 m μ) with Prednisolone Sodium Phosphate (λ_{\max} 257 m μ) in 5 percent Dextrose Injection at one hour	75
17.	U.V. Spectrum of Methotrexate Sodium (λ_{\max} 302 m μ) with Prednisolone Sodium Phosphate (λ_{\max} 257 m μ) in 5 percent Dextrose Injection at four hours	76
18.	U.V. Spectrum of Methotrexate Sodium (λ_{\max} 302 m μ) with Prednisolone Sodium Phosphate (λ_{\max} 257 m μ) in 5 percent Dextrose Injection at eight hours	77
19.	U.V. Spectrum of Cytarabine (λ_{\max} 272 m μ) with Methotrexate Sodium (λ_{\max} 302 m μ) in 5 percent Dextrose Injection at one hour . . .	79
20.	U.V. Spectrum of Cytarabine (λ_{\max} 272 m μ) with Methotrexate Sodium (λ_{\max} 302 m μ) in 5 percent Dextrose Injection at four hours . .	80
21.	U.V. Spectrum of Cytarabine (λ_{\max} 272 m μ) with Methotrexate Sodium (λ_{\max} 302 m μ) in 5 percent Dextrose Injection at eight hours .	81
22.	U.V. Spectrum of Sodium Cephalothin (λ_{\max} 236 m μ) with Cytarabine (λ_{\max} 272 m μ) in 5 percent Dextrose Injection at one hour .	84

Figure		Page
23.	U.V. Spectrum of Sodium Cephalothin (λ max 236 m μ) with Cytarabine (λ max 272 m μ) in 5 percent Dextrose Injection at four hours	85
24.	U.V. Spectrum of Sodium Cephalothin (λ max 236 m μ) with Cytarabine (λ max 272 m μ) in 5 percent Dextrose Injection at eight hours	86
25.	U.V. Spectrum of Sodium Cephalothin (λ max 236 m μ) with Cytarabine (λ max 272 m μ) in 5 percent Dextrose Injection at one hour	88
26.	U.V. Spectrum of Sodium Cephalothin (λ max 236 m μ) with Cytarabine (λ max 272 m μ) in 5 percent Dextrose Injection at four hours	89
27.	U.V. Spectrum of Sodium Cephalothin (λ max 236 m μ) with Cytarabine (λ max 272 m μ) in 5 percent Dextrose Injection at eight hours	90
28.	U.V. Spectrum of Sodium Cephalothin (λ max 236 m μ) with Methotrexate Sodium (λ max 302 m μ) in 5 percent Dextrose Injection at one hour	92
29.	U.V. Spectrum of Sodium Cephalothin (λ max 236 m μ) with Methotrexate Sodium (λ max 302 m μ) in 5 percent Dextrose Injection at four hours	93
30.	U.V. Spectrum of Sodium Cephalothin (λ max 236 m μ) with Methotrexate Sodium (λ max 302 m μ) in 5 percent Dextrose Injection at eight hours	94
31.	U.V. Spectrum of Vincristine Sulfate (λ max 256.5 m μ) with Methotrexate Sodium (λ max 302 m μ) in 5 percent Dextrose Injection at one hour	97
32.	U.V. Spectrum of Vincristine Sulfate (λ max 256.5 m μ) with Methotrexate Sodium (λ max 302 m μ) in 5 percent Dextrose Injection at four hours	98
33.	U.V. Spectrum of Vincristine Sulfate (λ max 256.5 m μ) with Methotrexate Sodium (λ max 302 m μ) in 5 percent Dextrose Injection at eight hours	99
34.	U.V. Spectrum of Vincristine Sulfate (λ max 256.5 m μ) with Cytarabine (λ max 272 m μ) in 5 percent Dextrose Injection at one hour	101

Figure		Page
35.	U.V. Spectrum of Vincristine Sulfate (λ max 256.5 m μ) with Cytarabine (λ max 272 m μ) in 5 percent Dextrose Injection at four hours	102
36.	U.V. Spectrum of Vincristine Sulfate (λ max 256.5 m μ) with Cytarabine (λ max 272 m μ) in 5 percent Dextrose Injection at eight hours	103
37.	U.V. Spectrum of Vincristine Sulfate (λ max 256.5 m μ) with 5-Fluorouracil (λ max 265.5 m μ) in 5 percent Dextrose Injection at one hour	105
38.	U.V. Spectrum of Vincristine Sulfate (λ max 256.5 m μ) with 5-Fluorouracil (λ max 265.5 m μ) in 5 percent Dextrose Injection at four hours	106
39.	U.V. Spectrum of Vincristine Sulfate (λ max 256.5 m μ) with 5-Fluorouracil (λ max 265.5 m μ) in 5 percent Dextrose Injection at eight hours	107

CHAPTER I

INTRODUCTION

Parenteral therapy is a means by which drugs, nutrients, and fluids may be given to a patient, usually through a vein or into a muscle, or more precisely, "under or through one or more layers of the skin or mucous membrane (1)." The growth and development of parenteral therapy, like many other procedures in medicine, have progressed slowly and erratically throughout its history. Advancement has been stimulated, in many cases, through a basic need for a new and better way. An excellent example of this is the fairly recent development of intravenous hyperalimentation by Dudrick (2).

Intravenous therapy followed the discovery of the circulation of blood by Harvey when Sir Christopher Wren attempted to give intravenous injections of medication to dogs, apparently with some success (3). Little did he know of the tremendous field into which parenteral therapy would develop.

Probably the first recorded successful transfusion occurred in Paris by Denys in 1666. This transfusion into a human being was done using the blood of a calf to treat an idiot, at the request of the idiot's wife. The first successful experimenter to transfuse blood from one human

being into another was Blundell of England in 1824. Blundell believed that small transfusions of blood might save some of his cases of severe post-partum uterine hemorrhage which usually terminated in death (4).

In Edinburgh, in 1832, during a cholera epidemic, Latta, in a desperate attempt, administered a solution of sodium chloride and sodium bicarbonate to his patients. The dramatic improvement that resulted initiated the real start and development of intravenous therapy (5). Parenteral administration, particularly intravenous therapy, was originally pioneered in the United States in the middle 1800's and developed steadily as new concepts of administration, accuracy of dosage, and better analytical methods for the investigation of body fluids were developed (6).

The benefits of intravenous therapy have become more and more apparent over the years. Medications can be given rapidly with an expectant rapid onset of action. The response to the drugs or fluids can often be closely controlled by regulating the dose or rate of administration. Frequently, adequate blood and tissue levels needed to eradicate many serious infections can be reached only by this route. Intravenous therapy is an especially appropriate method when the use of the oral tract, for one reason or another, cannot be used.

The development of intravenous therapy, however, did not proceed without its difficulties. Problems of allergic reactions, incompatible blood groups, bacterial

contamination, particulate matter, thrombophlebitic syndromes, stability of solutions, and incompatibilities of admixtures soon became apparent. The purpose of this paper is to explore certain aspects of the latter problem, i.e., intravenous incompatibilities.

Problems of Intravenous Admixtures

An incompatibility has been aptly defined by Parker (7) as, "the failure of a drug or drug mixture to combine with another drug in an expected or desired manner." Useful information about intravenous incompatibilities is only slowly being developed, both for investigational and commercially available drugs. The reasons for this are probably many. It would appear that manufacturers would have accepted the challenge of gathering this information, at least in respect to their own products. However, the problems of intravenous solution incompatibilities of drug additives consist of the problems of two or more drugs interacting with each other physically, chemically, or therapeutically in one or more different infusion fluids. When drugs other than a manufacturer's own products are involved, the problems of liability concerning another company's drugs comes into focus. While the manufacturer may have information on his product, he may not know what other drugs a physician will prescribe to be in an admixture with his drug. Can one manufacturer assume the responsibility of another manufacturer's products? According to Superstine (8), the answer appears to be no, in that,

"manufacturers of drugs intended for intravenous use may be responsible only if and when their preparations are used according to their instructions." In an attempt to answer, or rather avoid, the problem of intravenous incompatibilities, a number of drug companies have advised, in their product information booklets and package inserts, that their parenteral products be used immediately after reconstitution and that the admixture of parenteral medications be avoided whenever possible (9).

Another problem that should be considered with regard to the manufacturers and the question of intravenous admixtures is their reluctance to supply vital information to other researchers working in the area of admixture compatibilities. Latiolais (10) states that when a pharmacist writes a letter with a question to a pharmaceutical company, the letter is often referred to the legal department because the company is concerned about the problems that it may encounter with the F.D.A. in giving an answer. He explains that, instead of going to the scientific division of the company, initial drug inquiries often end in the legal office because of the restrictions the government puts on them concerning distribution of unapproved drug information. Many times, the net result is no answer. The company explains that it cannot give the information without having it cleared through the "red tape" of the F.D.A. In response to the comments of Latiolais, Sleezer (10) of Hoffman-LaRoche states that, "perhaps ninety per cent of the things that you

do as a hospital pharmacist is legally putting the pharmaceutical manufacturer in the position of having an N.D.A. on their hands when they give you any comment." If a manufacturer disseminates, either privately or publicly, information regarding the compatibility of its products with those of another, the situation might be considered tantamount to promoting a new product. Such admixture combinations might come within the legal definition of new drugs and require the submission of extensive laboratory, animal, and clinical studies. Meyers (11) states that; "it would be an impossible burden for each manufacturer to carry out such studies for every possible combination of his particular preparation with other injectables."

Thus, only to a very limited extent, have manufacturers accepted the responsibility for determining the compatibility of intravenous drug admixtures. The few companies that have contributed work in this area have been, for the most part, those that manufacture the various infusion fluids. One of the better examples of this is the work that resulted in the charts published by Abbott Laboratories^a which describe compatibilities and incompatibilities of drugs in their solutions. The early work done in developing these was by Kirkland (12), who conducted his studies on behalf of the company. The main disadvantages

a - Abbott Laboratories, North Chicago, Ill.

of these charts, as well as the other early charts and tables developed from the work of the few companies and the individual private investigators, are especially apparent when one attempts to extract some useful information from them. Not only are they seriously deficient with regard to specifics about the admixtures but, for the most part, they include only information concerning so-called ~~physical or visual incompatibilities~~. Because of this, where combinations have been designated as "compatible," Webb (13) believes that the word "soluble" would be more appropriate. Usually there are no claims made regarding chemical or pharmacologic compatibilities. Meyers (14) admonishes; "when compatibility tables and charts are prepared. . . without evaluating either the pharmacological or chemical interactions. . . the information is in itself a possible hazard to the patient who will receive the extemporaneously mixed injection."

To add to this problem, the authors of the charts and tables usually summarize with an attempt to discharge any liability for the information by enclosing a disclaimer. While this may be consistent with good legal practice, it does not appear that these escape clauses are the answer to the manufacturer's responsibility (15). The one good attribute of the various charts is that there has been an attempt to centralize the available data regarding the physical incompatibilities into a more workable form.

Another reason for the lack of information on

intravenous incompatibilities is probably because of the enormous number of possible admixture combinations. The combination of drugs extemporaneously tailor-made at the direction of the physician is extensive. Using a computer, in 1965 Dunworth and Kenna (16) determined that for the 24 most commonly used medications which were added to intravenous solutions at their hospital, over 11,000 unique combinations in pairs, threes, and fours were possible.

Misgen (17) noted that with a given 500 drugs, 124,775 different combinations of two drugs each were possible. He continued that, if these 500 drugs were mixed in combinations of more than two, then the number of possibilities becomes astronomical. According to Plein (18), if 100 drugs are cross-matched three at a time, 161,741 different combinations are possible. These figures alone are immense. The other possibilities of mixing the drugs in the various different commercial solutions available, not to mention different doses of the various drugs, must then be superimposed on those statistics.

When one considers that even for one particular drug, the formulation may differ from manufacturer to manufacturer due to the addition of different buffers, solubilizers, preservatives, and anti-oxidants, which also can contribute to a chemical or biological incompatibility, another complication is introduced. This is especially true when one considers commercially available trade name or proprietary products in contrast to the generic or

non-proprietary brands that are used more and more commonly. Recently, studies are becoming available to confirm the belief that products from different manufacturers that were once claimed to be generic equivalents, are in fact not equivalent due to differences in adjuvants used and in methods of manufacturing. Wagner (19) has recently listed the 12 currently controlled studies in which two or more commercial drug products, containing the same drug in the same type of dosage form and compared in man, are summarized. Large statistically significant differences between a particular product manufactured by different companies were sometimes quite apparent. The differences may have been the result of different adjuvants used in formulation of the products, as well as different methods of production employed.

Another problem to be considered is that, even among a manufacturer's own products, a specific product may differ from lot to lot. A good example of this was illustrated in "Clin-Alert" (20). Certain physicians in Australia were having difficulties with some of their patients on diphenylhydantoin. These patients were experiencing signs of overdosage and no reason could be given until it was found out that the manufacturer had changed the excipient present in the capsules manufactured in Southeast Asia. This change caused a greater percentage of the dose to be absorbed, yielding higher blood levels of the drug followed by signs of toxicity.

Variations among a company's own products or those of different companies frequently result from the lack of rigid specification in the official monographs that set standards for most products available. The official monographs do not specify what adjuvants may or may not be used in the dosage form of a drug, nor do they specify any particular manufacturing methods. Thus, it is no surprise that products can differ widely from manufacturer to manufacturer.

With the advent of increased implementation of centralized intravenous additive programs, a service which complies with Accreditation Standards set by the Joint Commission on Accreditation of Hospitals (21), orders from physicians for intravenous admixtures are receiving closer scrutiny and evaluation. Few physicians or nurses have sufficient knowledge of chemistry to appreciate all possible risks of mixing drugs in parenteral infusion. Neither do they have the understanding of the somewhat scanty information that is available from manufacturers about the behavior or loss of activity which may follow injection of substances into various intravenous solutions (22). The sparse data published on stability of drugs in intravenous solutions have appeared almost solely in pharmaceutical journals, rarely read by doctors or nurses. One must appreciate that they have not kept abreast of all the problems and risks involved in adding one or more drugs to an infusion fluid. This should be the responsibility of the pharmacist who

must assume this compounding function.

As might not have been the case a few years ago when the nurse interpreted the physician's orders and then proceeded to add whatever medications were called for into the I.V. bottle, today's orders are being reviewed more closely for incompatibility and instability before admixture by the pharmacist. In order to perform an intravenous additive service from the standpoint of safety and economy, the pharmacist must have reliable incompatibility literature available. It is of interest to note that, according to Peritore (23), since the implementation of their centralized intravenous additive program at their hospital, an average of three intravenous incompatibilities or unstable mixtures have been avoided each week.

As has been alluded to before, many investigators in the past have tried to classify intravenous incompatibilities into various categories with such labels as "therapeutic," "physical," and "chemical." While these classifications are of use, the real emphasis in the literature should be on whether, in the end, the desired therapeutic response will be altered. The line, if there is one, dividing physical and chemical incompatibilities is often very narrow. A physical incompatibility is simply a chemical incompatibility that can be seen with the naked eye. Originally, physical incompatibilities implied to those in which the physical properties of the ingredients produced a mixture that was unacceptable in appearance only.

The overall objective, again, should be to decide whether the therapeutic effect of the drugs involved is altered and whether the safety and purity of the intravenous medication is retained following the mixing of the drugs. Academic categorization is of interest, but the physician wants to know if the combination is safe and still clinically effective.

One approach to the problem of intravenous incompatibilities in parenteral admixture might be simply to discourage the mixing of drugs so as to avoid the problem. While this may be a valid concept, it is not a realistic approach and has not been routinely accepted in the practice of medicine. It is an unfortunate fact that an ill person does not always have only a single symptom which can be treated with a single drug. Often there are other factors that complicate the situation. Therefore, it is not surprising that the physician will often attempt to mix two or more drugs in order to more effectively control the several symptoms with which the patient presents. In certain cases, the patient's condition may require that the total amount of fluids be limited (24), or the physician may not want to inconvenience the patient with another venipuncture (25). Under these circumstances, the drugs must be administered to the patient within the volume of solution available for use. Still, in other cases, for the required drugs to be administered, they must be mixed for sake of convenience and practicality. Extemporaneous

mixtures of drugs will invariably bring with them the risk of drug incompatibility.

That drugs are indeed added to intravenous solutions in various combinations is a reality; polypharmacy is still widely practiced. One need only visit a surgical or medical ward of a hospital to discover this. The literature is replete with numerous surveys taken by pharmacists in order to determine the extent that drugs and combinations of drugs are added to parenteral solutions in certain hospitals.

Tables I and II summarize a few of the studies that have been done in recent years. Table I indicates the percentage distribution of I.V.'s ordered with drug additives according to the number of additives. Table II demonstrates the frequency of distribution of drug ingredients added to all I.V. solutions.

A three months analysis of intravenous admixture orders in a teaching hospital revealed that there were, on an average, 1.15 additives per infusion bottle (38). In the study performed by Holysko and Havin (26), it was found that an average solution for administration would contain 1.9 drug additives.

A booklet entitled, "I.V. Additives: Steps to Safety" (39), prepared to serve as a guide for those either starting or wanting to improve an existing intravenous additive program, includes the following interesting statistics:

1. Sixty-three (63) million I.V. prescriptions are administered in the United States each year.

TABLE I

Frequency of Additives Included in Intravenous Solutions

(Percentage Breakdown Includes Only Those I.V.'s Which Contained Additives)

Study	Year	No. of I.V.'s	1	2	3	4+
			% of Solutions			
Holysko & Ravin (26)	1965		48	30	19	3
Ho & Rosero (27)	1965	(138)*	30	24	14	32
Meisler & Skolout (28)	1966		58	27	9	6
Patterson & Nordstrom (29)	1968	1,172	40	24	12	11
Pang (30)	1970	701	76	19	3	2
Reifman (31)	1971	1,552	75	20	5	0
Petruconis & Newman (32)	?	226 (141)*	58	38	4	
Petruconis & Newman (32)	?	(890)*	54	24	12	9
Average			52	26	10	9

*I.V.'s with additives only.

TABLE II

Frequency of Additives Included in Intravenous Solutions
(Percentage Breakdown Includes Number of Additives in All I.V.'s Surveyed)

Study	Year	No. of I.V.'s	1 or More	1	2	3	4+
			%	% of Solutions			
Holysko & Ravin (26)	1965		45				
Ho & Rasero (27)	1965	138	70				
Meisler & Skolout (28)	1966		50				
Goodwin (33)	1966		57				
Carlin & Perkins (34)	1968	716	65	46	14	4	1
Patterson & Nordstrom (29)	1968	1,172	86				
Jacobs & Superstine (35)	1970	500	60*				
Pang (30)	1970	701	60				
Francke & St. Clair (36)	1971		74	50	14	4	5
Reifman (31)	1971	1,552	51				
Catania (37)	1972	1,202	45	33	7	6	
Petruconis & Newman (32)	?	226	62				
Average			60				

*Sixty percent contained two or more additives.

2. Of these, 25 million I.V. prescriptions contain one or more additives.

3. The probability is that 1 out of 50 persons in the United States will receive an I.V. admixture in a hospital.

For the future, Donn and his colleagues (40) believe that intravenous admixtures containing more than one drug additive will be a technique of the past in the next few years. However, a habit of many decades cannot be broken overnight. He suggests that the percentage of admixtures containing only one additive has increased from approximately 50 percent to over 80 percent, although no substantiation is offered. He believes this to be a result of the education of the medical staff to the problems associated with intravenous admixtures. He speculates that "mini-bottles" containing one additive might be manufactured to provide "pulse therapy" via an intravenous Y setup to eliminate all admixtures containing a multiplicity of drugs.

It is very apparent from these few examples that the use of multi-component admixtures in various American hospitals is very prevalent.

Because work in parenteral incompatibilities has frequently been done on a "piece-meal" basis rather than on the basis of scientific deliberation, data have accumulated slowly. Early methods of incompatibility studies, as mentioned before, were often crudely accomplished in that judgments were made on the basis of visual observation. It

is also evident that the earlier methods were often hardly scientific in that they often lacked vital information such as drug concentration, type of parenteral fluid used, order of mixing, storage conditions, the volume of the vehicle used, etc.

There is a lack of uniformity in the methods that have been used to detect incompatibility and different methods have relied upon different guidelines for detection. Often the methods were somewhat unrealistic and did not simulate actual clinical practice.

Frequently, authors would attempt to recommend procedures to eliminate the problems that might arise. In order to resolve the problem of a precipitate, they might suggest either to mix the admixture well or to change the order of mixing. While this may eliminate or delay immediate formation of a precipitate, it is unlikely that the ingredients will be protected from chemical degradation. An example of this is the report by Jones (41) who stated that Solu-Cortef^a was compatible with Nembutal Sodium^b based on physical (visual) results. However, on the basis of the results of Anderson (42), this combination should be predicted to be incompatible (chemically) due to the high pH of the combination resulting from the alkalinity of the Nembutal Sodium.

a - Merck Sharp & Dohme, West Point, Pa.

b - Abbott Laboratories, North Chicago, Ill.

In addition to these weaknesses, Meyers (43) also adds the following points:

1. Many formulas actually tested have subsequently been altered.

2. Many combinations tested in advance by pharmaceutical manufacturers have never been prescribed and, more important, should never be prescribed.

3. ~~Compatibility testing programs are, for the~~
most part, superficial in nature.

Donn (40) expressed his discontent with the available information on intravenous incompatibilities, particularly the charts. His reasons were the following:

1. Conclusions are usually based on those additives manufactured by a single source and may not in all cases be chemically the same as the additive stocked by a particular hospital.

2. The solutions utilized may not in all cases be chemically the same as the solution utilized.

3. The criteria utilized in making a decision as to the compatibility of intravenous admixtures are not standardized.

4. Combinations are usually limited to two drug additives in a single I.V. solution.

5. Frequent conflicts between references exist listing identical combinations.

6. Data obtained from studies are often scientifically questionable.

7. The information is usually inadequate in that not all of the additives used in a particular hospital are listed.

To deal with the problems of possible drug interactions that may arise with the use of multi-drug admixtures in infusion fluids, many pharmacists and other workers have attempted to conduct various types of compatibility studies. The Public Health Service has recently made available a list of references on intravenous additives covering the years from 1955 to 1971 (44). It is quite apparent from their list of 133 references that there has indeed been a great deal of activity in this area. These studies may have utilized the very simple procedure of adding two or more drugs to a solution and observing the "physical," or rather the visible, results that might occur as determined by the presence of a precipitate, color change, evolution of a gas, or the presence of a haze or cloudiness. At the other end of the spectrum, procedures involving extraction or chemical alteration and manipulation of the drug after admixture and subsequently biological and chemical testing have been employed to determine its potency and structural integrity.

One of the earliest studies conducted was that by Bogash (45) in 1955. After admixture of specific parenteral products to commonly used solutions designed for intravenous injection, the solutions were inspected immediately and after four hours. He based compatibility on the absence of

particulate matter in the solutions and later compiled one of the first compatibility charts on the basis of his work. Bogash stressed that it was difficult to appraise whether several parenteral drugs were compatible upon admixture. It appears that he also exhibited a keen insight into the problem by observing that the constituents of the various solutions have become complex and, in many cases, the necessary adjuvants further complicate the final admixture.

In 1961, Kirkland and his associates (46) conducted a similar study. Their work remains today as one of the most extensive of all tests carried out representing more than 8,000 tests using 137 drugs singularly or in combination with 60 Abbo-Liter^a parenteral solutions. The results of their tests, which were based on visible results over 24 hours, were compiled into a comprehensive and complex compatibility chart. They realized that due to the multiplicity of ingredients there are many other reactions, whether visibly detectable or not, which may occur when parenteral medications are combined.

Riffkin (47) warned that extemporaneous mixing of drugs invariably brings the risk of drug incompatibility. He further noted that any changes in pH, viscosity, tonicity, or particle size distribution could easily upset delicate systems. In addition, modifications of oxidation or reduction conditions, light exposure, or storage temperature

a - Abbott Laboratories, North Chicago, Ill.

requirements could result in a loss of activity.

Later, Dunworth and Kenna (16) stated that very little information was available depicting the incompatibilities of two medications in intravenous solutions and even less information had been published indicating the effect of buffers, preservatives, etc., on the compatibility of medicaments in intravenous solutions.

In the same year, Misgen (17) announced the results of his study which were accomplished by adding one ml. aliquot of reconstituted additives to five mls. of sterile distilled water in pairs. The admixtures were then observed for signs of particulate matter. In this study, 34 drugs intended for intravenous use were cross-matched to test for visible signs of incompatibility. His comment that,

"... it might be safe to assume that if the drugs were incompatible at high concentrations, a similar phenomenon might be expected to occur in more dilute solutions in a high percentage of the situations,"

may be open to question.

In 1966, Im and Latiolais (48) reported on the results of their work which investigated the physico-chemical compatibility of admixtures of penicillins and tetracyclines in 5 percent Dextrose Injection. Their method to detect drug interactions was based on degradation evidenced by spectrophotometric analysis of the individual drugs. A loss in absorbance at the wavelength of maximum absorption formed a basis for adjudging the mixture incompatible.

Patel and Phillips (49) used the microscope in an

attempt to develop another method for studying physical compatibility of intravenous drug admixtures. The published results also lacked many of the specifics and details in terms of methods and techniques needed for properly understanding and interpreting their compatibility chart. The study was conducted simply by examining the admixture with a microscope for visible evidence of a precipitate.

In 1967, Gallelli (50), at the National Institutes of Health, conducted stability studies of six different drugs when added individually to intravenous infusion fluids. His study was limited to single component admixtures of the drugs to his infusion fluids of Sodium Chloride Injection and 5 percent Dextrose Injection. His methods of assay of the drugs, at time intervals of over a period of one to four weeks, included either spectrophotometric or microbiological assay. This study is the only one published in the literature which involved the stability and duration of activity of anticancer drugs. The two oncolytic agents that he worked with were mercaptopurine sodium and cyclophosphamide. Gallelli compared his results to the statements found in the manufacturer's product information concerning the administration and stability of the drugs in intravenous solutions and found that in many cases the drugs were stable for much longer periods of time than had been recommended.

During the same year, Parker (51), acting on behalf

of Abbott Laboratories in continuing the work of Kirkland, reported on a chemical compatibility study that involved eight drug products in commonly used intravenous fluids. Using pH readings and chemical or biological assays, he presented one of the first examples of chemical incompatibilities which did not produce visual changes in admixture. Parker emphasized the important role pH plays in the stability of a drug when diluted to a large volume by a solution of a different pH. These solutions may contain dissolved atmospheric oxygen which might catalyze or initiate chemical reaction and cause decomposition of the drug. Unfortunately, with this study, which is also true of later publications of Parker, experimental methods and procedures were not stated.

Methods of Predicting Intravenous Incompatibilities

The results of the experiments reviewed thus far were, for the most part, the results of actual experimentation and work with intravenous drug admixtures. In more recent years a number of authors have attempted to predict the pharmaceutical incompatibilities of parenteral medications which might occur. As will be shown, most of these predictions have been based on an alteration of pH when a drug is added to a vehicle of different pH. One of the earliest articles written concerning the prediction of intravenous compatibilities was that written by Edward (53). In her study, Edward determined the pH change in an intravenous

vehicle when a drug, or combination of drugs were added. This information was then compared with the known pH stability range of each drug. The results of this comparison could then be used to predict the possible acid-base stability of drug or drug combinations in a vehicle.

Carlin and Perkins (34), on the basis of a survey of patient charts at a local hospital, reviewed the potential inter-reactions of the penicillins and other drugs.

By using available data from chemical incompatibility studies, a method was demonstrated for estimating the reaction rate of certain additives by calculating the time required for 10 percent of the drug to react.

Later, Webb (53) attempted to show a relationship of incompatibility to pH by rearranging the compatibility chart compiled by Patel and Phillips so that the additives were listed in terms of increasing pH, and were at right angles to the same additives listed in terms of decreasing pH. On the basis of this chart, it was Webb's contention that intravenous incompatibilities could be predicted due to the fact that the solutions with extreme pH values are incompatible with solutions with pH values of the opposite extremity.

More recently, Ho and co-workers (54,55,56), in a series of articles, have attempted to demonstrate how to predict the pharmaceutical stability of parenteral solutions from the point of view of kinetics and particularly rate constants. While Ho explains that the "elementary"

mathematics used in his articles should present no problem to the present day pharmacist with an undergraduate training in physical pharmacy, it is this author's opinion that he does exceed the immediate comprehension level and point of practicality needed to reach the average hospital pharmacist. While Ho's work is probably beyond the mathematical ability of the average hospital pharmacist, he does make a few astute comments on the deficiency of present literature on intravenous incompatibilities. Such literature is presented in a manner that is not sufficiently comprehensive to relate the various situations such as pH, temperature, and vehicle. He explains his reasoning in the following:

"First, it takes time, effort, initiative, and reasonable scientific ability for a research-oriented hospital pharmacist to find a reliable analytical method and then to study the kinetics of decomposition of the drug under varying conditions. Secondly, the presentation of the data in a manner that can be understood and utilized by others to whom the study is intended may vary."

His papers attempt to help the professional pharmacist consolidate and interpret the current literature on the stability of clinically significant parenteral admixtures.

Recent Studies of Intravenous Admixtures

Work in the more recent years, in the area of intravenous incompatibilities has been somewhat of a different nature than the earlier studies. Earlier work concerned itself basically with the nature of physical incompatibilities and for the most part was conducted on the basis of a survey of drugs in general and drug classes. Detailed

involvement concerning individual incompatibilities was usually not attempted. The major emphasis was on covering a broad spectrum of drugs on a somewhat superficial basis, skimming the surface of the problem as it were. In contrast to this, the more recent studies have mostly limited themselves to the incompatibilities of one or two drugs in combination with a few other drugs. The newer studies have been more detailed in relating techniques and have attempted to discuss the individual reactions that might occur between drugs and the effect of these reactions on the efficacy and safety of the mixture. Attempt has been made, where possible, to examine the therapeutic integrity of a drug after it has been put in combination with other drugs.

The research conducted by Simberkoff, et. al. (57), is of particular interest because it is one of the only studies on the stability of drugs in infusion fluids conducted by physicians. In this study, the inactivation of different penicillins by carbohydrate solutions at alkaline pH presented a new concept of incompatibility. It is their finding that when penicillins are added to solutions of 5 percent Dextrose Injection, rendered to an alkaline pH, it is the carbohydrate fraction of the solution that promotes the breakdown of the penicillin moiety. They hypothesized that penicilloyl esters of the carbohydrates are formed initially. These then undergo hydrolysis under the conditions of the reaction mixture.

Another study involved the parenteral compatibility of admixtures containing metaraminol bitartrate and hydrocortisone sodium succinate alone and in nine combinations of various concentrations and at various pH values in 5 percent Dextrose Injection. That investigation included a complex assay procedure involving pH adjustments and extractions with chloroform with a final analysis using either an ultraviolet or infrared spectrophotometer (58).

In a study of the stability of ampicillin and carbenicillin in commonly used infusion solutions, Jacobs and co-workers (59) determined the potency of these antibiotics using a microbiological assay at different time intervals over a period of 24 hours.

Zost and Yanchick (60) determined the compatibility and stability of disodium carbenicillin in three commercial infusion solutions and in combinations with six other drugs. Carbenicillin was assayed microbiologically by the use of a cylinder plate assay using a strain of the organism Pseudomonas. They found that carbenicillin is quite stable when added with other ingredients. It is of particular interest that in their study they found there was no correlation between changes in pH and changes in microbiological activity.

Riley (61) reported on a study on the visible effects of intravenous drugs added together in two standard intravenous fluids. This study very closely paralleled earlier studies done in the United States, that of Kirkland (12)

done ten years earlier.

Using an old principle and applying it in a novel manner with an innovative technique, Catania and King (62) presented their results of studying the physical and chemical compatibility of sodium ethacrylate when combined individually with eight cardiovascular and psychotherapeutic agents in Sodium Chloride Injection. Their adaptation involved the use of a spectrophotometer along with pH measurements to analyze the compatibility of the admixtures. They also found little correlation between the pH values of the admixtures and the nonvisual chemical reactions detected in their study.

Over the past three years, in a continuing series of articles, Parker (63) has attempted to relate the results of his continued work in the area of intravenous incompatibilities in Journal's department entitled, "Compatibility Digest." These are concerned with the compatibility of one particular drug in combination with other additives. The results are usually broken down into three tables:

1. Physical compatibility of the drug with other additives in I.V. admixtures;
2. Stability of the drug in various I.V. fluids;
- and
3. Stability of drug in dextrose 5 percent at various pH values.

It is unfortunate that the method or technique of drug assay is not well described.

As can be seen, much work has been done in the area of intravenous incompatibilities. Unfortunately, very few of the methods employed in the various studies have been standardized and often the results may not be entirely reliable. It has often been said that misinformation is worse than no information; however, because of the desperate need for such material, studies must be continued so that more and more information will be available to the practicing pharmacist and nurse who must then weigh and evaluate the studies as to accuracy and relevancy.

In an attempt to consolidate the many charts available and the numerous published and unpublished reports in the literature into a concise and usable form, King (64) has compiled a "Guide to Parenteral Admixtures." This represents the first successful attempt to centralize available data and to establish a mechanism for dissemination of updated material.

Oncolytic Drug Admixtures in Intravenous Chemotherapy

The term cancer includes a group of neoplastic diseases which occur in all races of man and species of animals. It is, in essence, a disease of cells characterized by a reduction or loss of effectiveness of the normal cellular control and maturation mechanisms which regulate multiplication and other functions required for homeostasis in a complex multicellular organism (65). In the United States, cancer rates second to heart disease as the major cause of

death, with over 300,000 fatalities a year. Under current treatment methods, only one-third of the patients are cured by surgery or radiation therapy. For the most part, chemotherapeutic agents have been employed toward the palliation of symptoms, management of complications, and prolongation of life (65). It should be emphasized that while chemotherapeutic cures are currently attained only in relatively rare tumors (e.g., choriocarcinoma and Burkitt's lymphoma), drug research may provide the ultimate cure for cancer. The future treatment and control of cancer will probably reside in the chemical approach, based upon a complete comprehension of the carcinogenic process (66).

As the incidence of cancer in the United States continues to increase, so does the use of those drugs used in the treatment of the disease. Since World War II, the number of anticancer drugs has increased tremendously. Unquestionably, the last 25 years have witnessed greater advances in this field than during any previous period (66).

Experimentation continues with the use of cytotoxic drugs. With the advent of the testing of new oncolytic agents and the new uses of older drugs, the field of cancer chemotherapy has become a very complex specialty. It is of interest to note that at the United States Chemotherapy National Service Center, approximately 30,000 substances are annually screened for anticancer activity (67). The parenteral use of anticancer agents, whether it be by the traditional intravenous route, or by less commonly employed

routes such as intraarticular or intrathecal, plays a large part in the use of these drugs (67). This is apparently so because many of the drugs are either not effective orally, or adequate blood levels cannot be achieved by the oral route. Also it may be that the specific site or target cannot be hit adequately without causing undue toxicity to the rest of the body. This may be the case in regional therapy and in intraarterial infusions (68).

In some circumstances, attempts have been made to improve the effectiveness of chemotherapy without excessive increase in toxicity, utilizing drugs in combination or sequentially (69). In treating the various types of cancers, the term combination chemotherapy implies that a given course of therapy consists of several drugs, chosen to exhibit varying mechanisms of action and varying manifestations of toxicity. These are administered according to a schedule devised empirically. This is an attempt to arrest as many neoplastic cells as possible in the dividing (drug-susceptible) stage, or to expose those cells to maximal toxic effects, but to limit or spread the side effects and toxicities of each drug to the patient over several organ systems (70).

Combination therapy is often employed and is of greatest value in tumors which grow rapidly and which produce resistant strains rapidly (71). In the treatment of leukemia, where the final objective is eradication of the disease, combinations of drugs have been used frequently

in an attempt to eliminate more of the leukemic cells. Although most of these procedures are still investigational, encouraging results have been obtained from multidrug regimens in which patients are treated with combination regimens variously known as VAMP, BIKE, or POMP treatments. The combinations of treatment consist of courses of vincristine, methotrexate, mercaptopurine, prednisone, and cyclophosphamide given at regulated intervals until a remission is obtained. In these treatments, because each drug is given in full doses, the possibility of toxic reactions is enhanced (68). Also, recently, combinations of drugs are proving to be more effective than single agents in inducing more complete remissions and longer durations of disease-free remission in such cancers as choriocarcinoma, Hodgkin's disease, acute lymphoblastic leukemia in children, reticulum cell sarcoma, and teratoma of the testis (72).

With the increased parenteral use of anticancer drugs, particularly those that are given intravenously as an infusion over a period of time, much concern has been generated about the stability and compatibility of these drugs in intravenous fluids, alone, and more particularly in combination with other anticancer or non-anticancer drugs.

In most teaching hospitals and government cancer institutions, the actual physical combining of anticancer drugs is discouraged. For an example, at two branches of the National Institutes of Health (the Clinical Center in Bethesda and the National Cancer Institute in Baltimore,

Maryland), the combining of anticancer drugs, as well as the combining of almost all drugs in intravenous mixtures is avoided. The principle reason for this is to avoid any possible chance for an interaction to occur that might alter the effect of their experimental drugs. Since these hospitals are basically research institutions, experimenting with new concepts and drug treatments, they simply do not want the possibility of any added drugs enhancing, decreasing, or nullifying the effect of the drug being studied (73). Another fact considered, specifically in regard to the anticancer drugs, is that most of these chemotherapeutic agents exhibit a very low ratio of therapeutic to toxic activity, and alteration of the drug in any way may seriously alter this delicate ratio and affect the outcome of the treatment. Doses of most of the oncolytic agents approach the limits of normal cell tolerance by design, so that maximal kill or inhibition of neoplastic cells occurs and the chance of survival of neoplastic precursors of the original tumor and any metastasis is minimized (70). Since each antineoplastic agent must be used in the optimal schedule, route, and dosage for the particular indication, any alteration in the agent could seriously decrease its effectiveness or cause serious toxicities and even death (68).

That oncolytic drugs are given in combinations and with other drugs in parenteral solutions appears to be a very prevalent fact, although probably not to the extent

as it is with other admixture combinations. In reviewing three of the main journals that are exclusively devoted to the subject of cancer, "Cancer," "Cancer Research," and "Cancer Chemotherapy," it is quite evident that combination intravenous therapy is indeed used. However, little information could be found regarding the compatibility of chemotherapeutic agents alone or in combination in infusion fluids. As mentioned before, Gallelli (50) studied the compatibility of mercaptopurine and cyclophosphamide alone in infusion fluids, but no attempt was made to determine the compatibility of the admixture of these two or other anticancer agents. Correspondence with selected companies requesting information on the intravenous admixture compatibilities of their oncolytic drugs, yielded the response that such information was not available. The only information that could be located on this subject was found in the booklet of compatibility tables of drugs in infusion fluids compiled by the Pharmacy Department at the National Institutes of Health (74). That compatibility data, however, is not documented and contains no references. For most of the anticancer drugs included in that booklet, a statement is made to the effect that, "It is recommended that no other drug be physically combined with this drug in the same infusion bottle because of lack of compatibility data." Thus it is quite apparent that essentially no work has been done in this area of intravenous incompatibilities.

It has been mentioned before that because of the

pharmacist's training and knowledge of chemicals and drugs, pH concepts, buffer systems, etc., he may, in many instances, predict potential "physical" and chemical, as well as therapeutic, intravenous incompatibilities. However, in other instances the pharmacist's logical predictions will not hold true and he must rely on the specific work of others, if such is available, in order to justify an admixture in terms of efficacy and safety. Investigational studies covering the physical and chemical compatibility of intravenous admixtures of chemotherapeutic agents, carried out on a scientific basis, is definitely needed. It is because of this that a study into the intravenous stability and compatibility of selected anticancer admixtures was initiated. It is perfectly clear that there is no short cut to taking the actual drugs themselves and mixing them with the infusion fluids and then analyzing the products to determine the effect on the components.

Because of the lack of information relating to the compatibility of antineoplastic agents, it is important to examine these as they might be prescribed for intravenous administration under clinical conditions. This study will include delineation of the physical and chemical compatibility characteristics of a group of agents which may be administered in intravenous admixture. Limitations in the selection of the specific drugs were imposed by the usual route of administration and availability of products. Investigational drugs were not considered in this work.

The four oncolytic agents to be examined include:

5-fluorouracil, methotrexate sodium, cytarabine, and vincristine sulfate. Because, on occasion, sodium cephalothin or prednisolone sodium phosphate may also be admixed with these, it seemed appropriate to include these as part of this study. Evaluation of admixture compatibility will utilize both visual and spectrophotometric procedures.

CHAPTER II

EXPERIMENTAL PROCEDURE

The purpose of this study was to determine the physical and chemical compatibility as well as stability of certain antineoplastic, antibiotic, and corticosteroid drugs in an intravenous infusion fluid. Five percent Dextrose Injection was selected as the vehicle because of its wide usage in medicine for the purpose of administering drugs. It was within the purposes of this study to simulate actual clinical practice as much as possible to provide results as realistic and meaningful as possible.

Physical compatibility was determined by visual observation of the admixture as might be manifested by the presence of a precipitate, color change, gas evolution, or by the presence of a haze or cloudiness in the solution. Chemical compatibility was determined through the use of spectrophotometric analysis, according to the method developed by Catania and King (62).

The drugs chosen for this study included:

5-fluorouracil (Fluorouracil)^a, sodium methotrexate

a - Roche Laboratories, Division of Hoffmann La Roche, Nutley, N. J.

(Methotrexate)^a, cytarabine (Cytosar)^b, vincristine sulfate (Oncovin)^c, prednisolone sodium phosphate (Hydeltrasol)^d, and sodium cephalothin (Keflin)^e.

Preliminary Study

A Bausch and Lomb Spectronic 600 Double Beam Spectrophotometer^e was used to obtain the ultraviolet absorbance spectrum of each of the drugs used in the study, uncombined and in the admixtures. The deuterium lamp of the spectrophotometer provided an electromagnetic incident beam in wavelength range of 220 to 350 millimicrons for the analytical absorption spectrophotometry. The solutions were analyzed in Bausch and Lomb 33-27-25 Silica Cuvettes and a Linear/Log Varicord 43 Recorder^f was used to obtain a recording of the spectra.

The dextrose injection^g used in this experiment was transferred throughout the experiment by means of an intravenous injection set.^h

-
- a - Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.
 - b - The Upjohn Company, Kalamazoo, Mich.
 - c - Eli Lilly and Company, Indianapolis, Ind.
 - d - Merck Sharp & Dohme, Division of Merck & Co., Inc., West Point, Pa.
 - e - Bausch and Lomb Optical Co., Rochester, N. Y.
 - f - Photovolt Corp., New York, N. Y.
 - g - Cutter Laboratories, Berkeley, Calif.
 - h - Saftiset #360-35, Cutter Laboratories, Berkeley, Calif.

In order to reduce any chance of error and to insure accuracy, before each spectrophotometric scanning or absorbance reading was done the instrument was calibrated. Every attempt, however, was made to standardize procedure and technique throughout the experiment.

In the preliminary work, Beer's Law curves were plotted for each of the drugs, added separately to the 5 percent Dextrose Injection. This was accomplished by preparing random dilutions of the drugs in the dextrose solution until a concentration was found where an absorption spectrum could be obtained. Dilutions were prepared to achieve maximum absorbance in the range of 0.2 to 0.9. In these, 5 percent Dextrose Injection was used as the reference solution. These spectra were taken to establish the reference or standard spectra for each of the drugs.

Analyses of Admixtures

For an analysis of the drugs in admixture, the six drugs were added in pairs to the 5 percent dextrose solution. These were cross-matched so that every possible unique combination would be tested. It was realized that probably not all combinations tested have been used in clinical medicine; however, it was felt that since the area of cancer chemotherapy is continually changing, the possibility still exists that all combinations might be used at some time or another.

Maintaining a constant pattern for the order of

mixing, the drugs were added to the solution of 5 percent Dextrose Injection. Thorough mixing was done after the addition of each drug; the final admixtures being stored at room temperature and with normal exposure to light.

For the purposes of determining the presence of any "physical" incompatibilities, the admixtures were observed for visual changes (color change, evolution of gas, precipitate, etc.) 1, 4, and 8 hours after initial admixture.

In order to determine the possible presence of any chemical incompatibility, the ultraviolet absorption spectrum for each drug while in combination was determined to observe any change in absorption characteristics. The resulting spectrum was then compared to the standard spectrum which had been obtained both in the preliminary study and at the start of each admixture. Any changes in the spectrum of a drug might present as an alteration in the number or size of the absorption peaks or as an appreciable change in the wavelength of peak spectral absorbance (λ_{\max}) of the drug.

Since each combination admixture contained two drugs in the 5 percent Dextrose Injection, in order to obtain a spectrum for one of the drugs present, everything in the combination, except the particular drug being examined would have to be blanked out. This was accomplished by making up two individual solutions each containing only one of the drugs in the pair. These were then used as the

reference solutions. Therefore, the composition of the reference solutions was identical to the admixture solutions except only one drug of the admixture pair was present. By simply alternating the reference solutions, individual absorption spectra could be obtained for each drug in the admixture solution. Each admixture was performed in triplicate and absorption spectra were obtained for each drug in the combinations at 1, 4, and 8 hours after initial admixture.

In order to simulate the actual conditions of admixture in the clinical setting, all drug preparations were reconstituted, if necessary, according to the manufacturer's recommendations. Appropriate quantities of the drugs were transferred to the infusion fluids with either graduated or volumetric pipets to 50 ml. volumetric flasks. The concentrations of the drugs used in the admixtures were selected to resemble, as closely as possible, those used in actual clinical practice. It was decided to use those concentrations that were considered, according to literature and the manufacturer's product information, normal doses for an average adult of 70 Kg. (154 lb.). The actual dosage of anticancer drugs employed frequently depends upon the particular type of cancer being treated. In this study, if this were the case for a particular drug, an attempt was made to use a representative concentration of the drug. Where it was found that a wide dosage range existed, the concentration used was that considered most convenient for

the spectrophotometric analysis. Since it was observed in the literature that most recommended doses were stated in terms of mg./Kg./day, a total daily dose for a 70 Kg. patient was calculated and divided by three. This amount, added to 500 ml. of the infusion fluid, yielded the concentration at which the experimental admixtures were prepared. For experimental conveniences, solutions and admixtures were prepared in 50 ml. volumetric flasks.

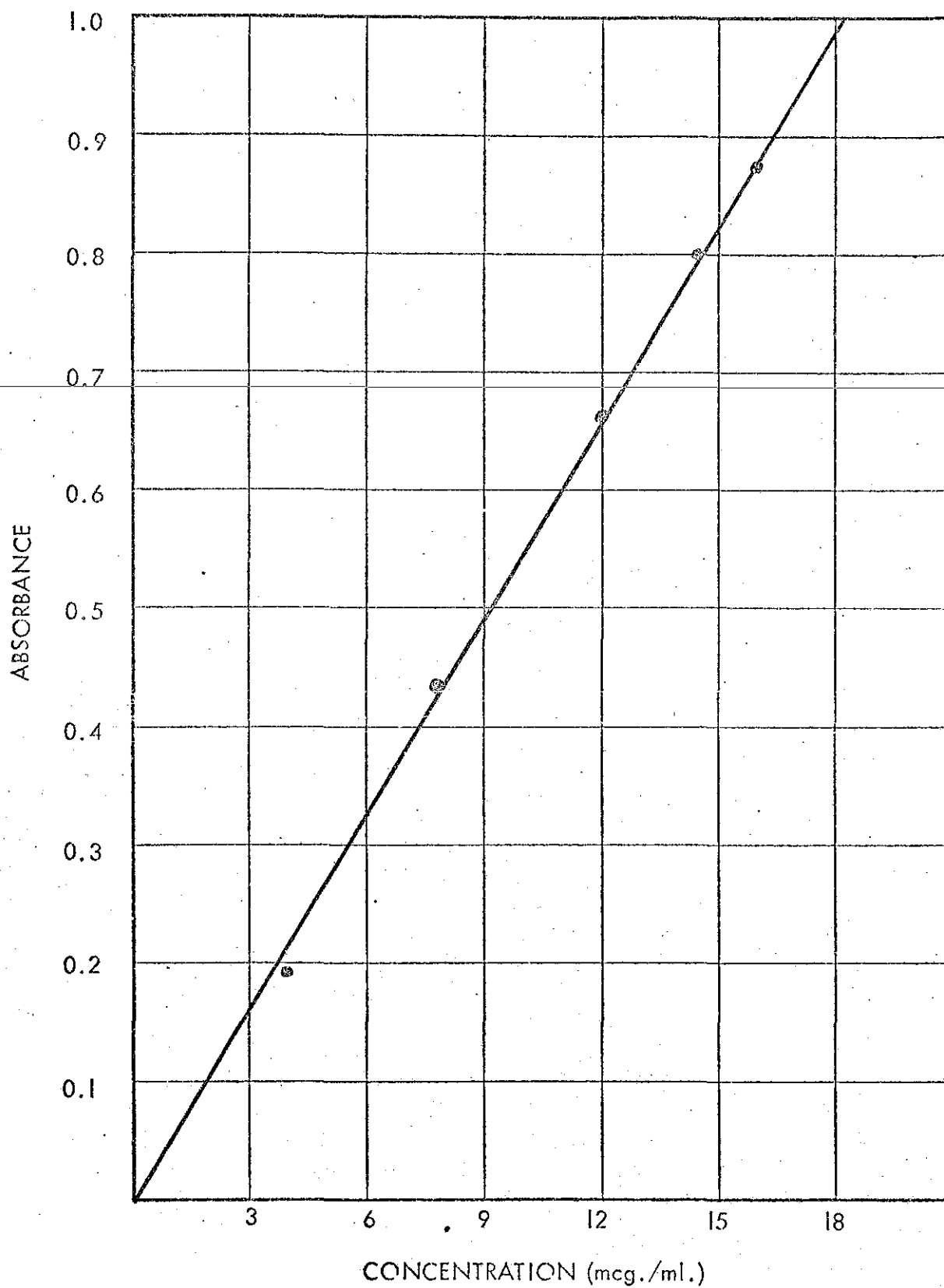
Although all admixtures were made at therapeutic concentrations, it was occasionally necessary to dilute the admixture immediately before scanning, to bring it into the concentration range which could be handled by the spectrophotometer.

CHAPTER III

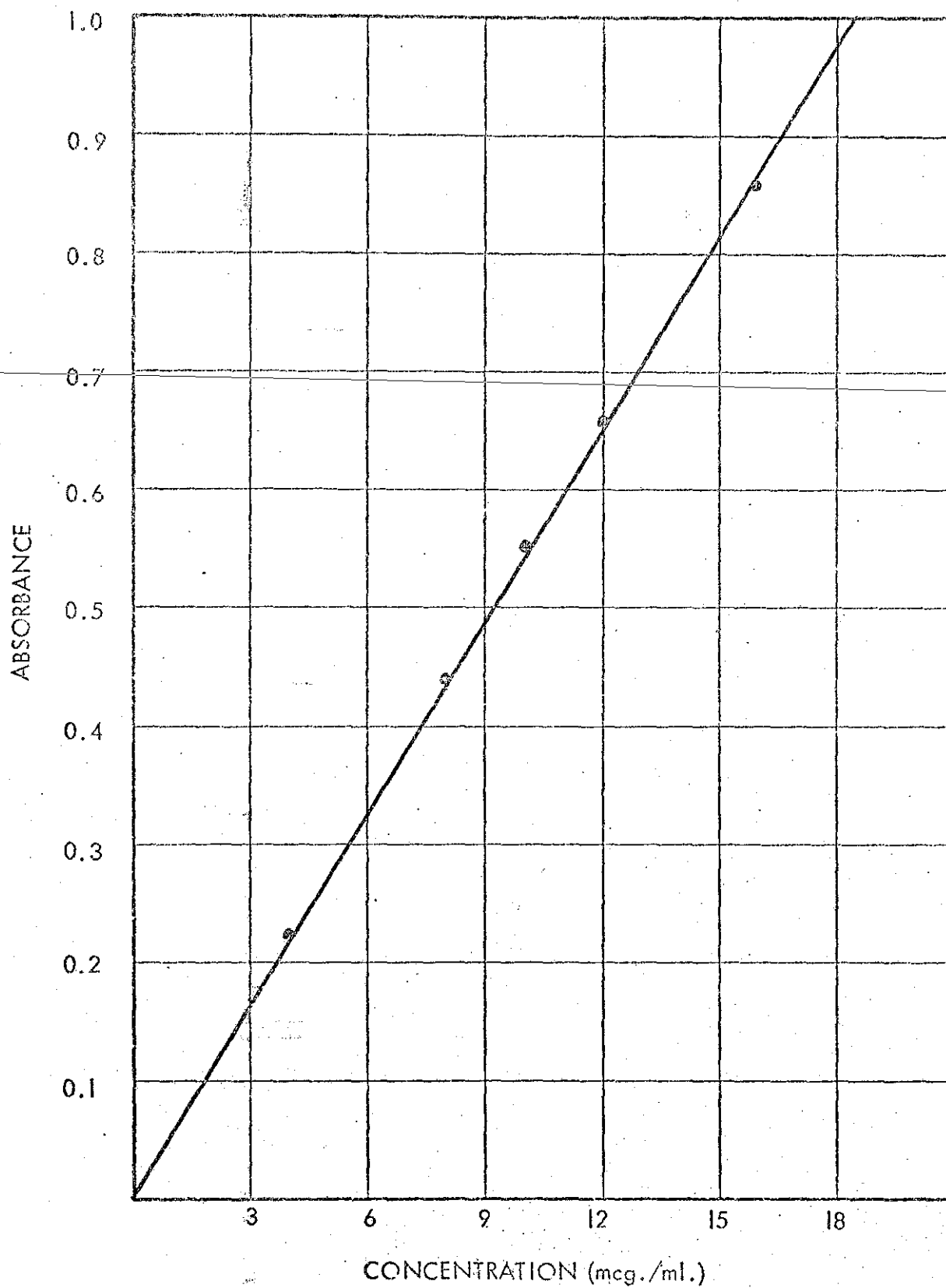
RESULTS

In the following section, a number of graphs displaying the Beer Law plots for each of the drugs, alone, in the 5 percent Dextrose Injection are presented (Graphs 1 through 6). These relate the absorbance at the λ_{\max} as a function of concentration.

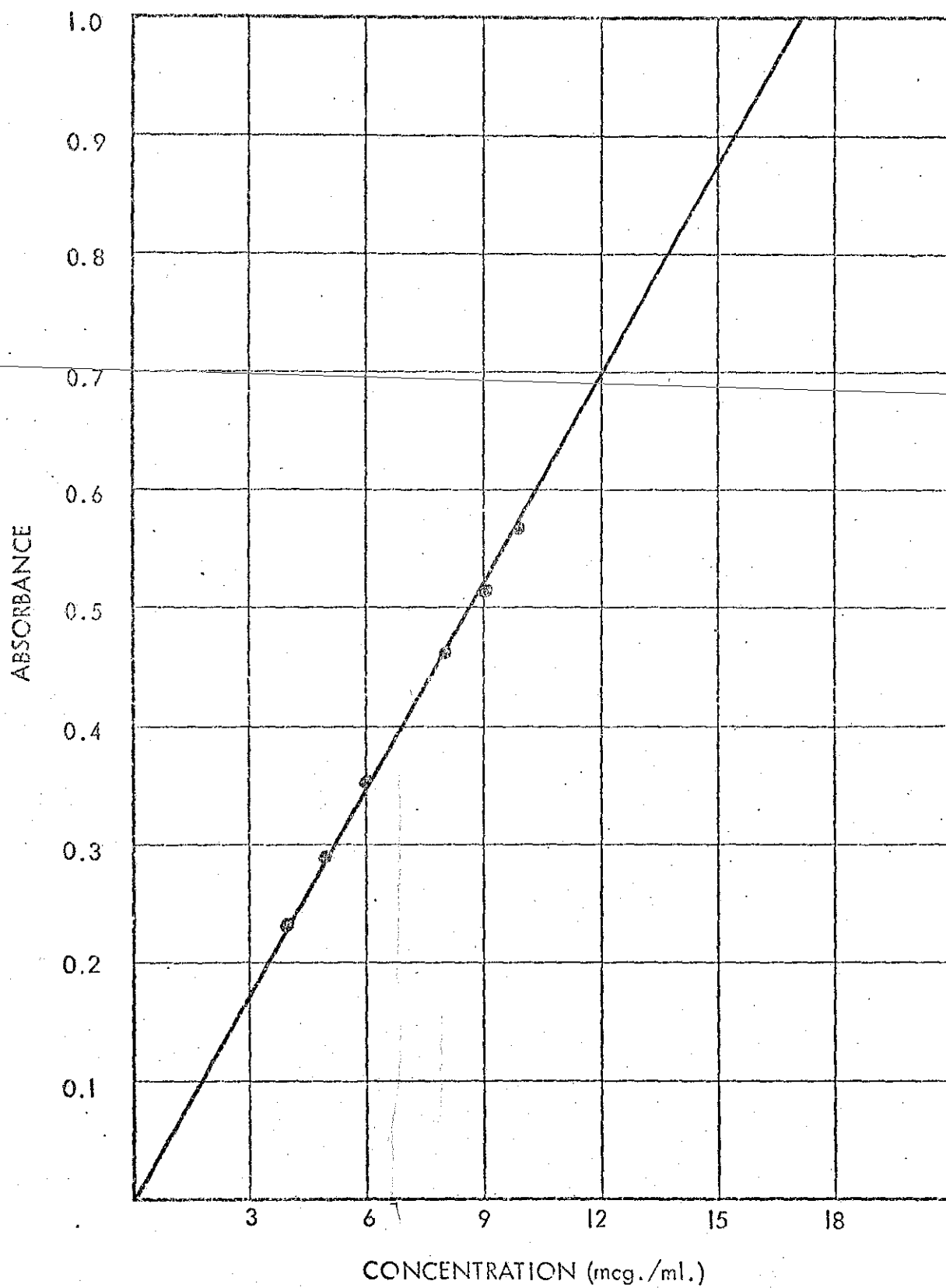
Figures 1 through 39 represent the results of the spectrophotometric analysis of each of the drugs, alone and in pairs as the admixtures. By comparing the spectrum of each drug alone with the spectrum obtained of the drug in combination with another drug, it was possible to determine whether a "chemical" (i.e., nonvisual) incompatibility might have occurred as a result of the admixture. Any alteration in the shape or structure of the spectrum, such as the addition or deletion of a peak, was considered to represent a probable chemical incompatibility. By comparing the magnitude of absorption at the wavelength of maximum absorbance with the Beer's plot for the drug, it was also possible to determine whether the potency of the individual drugs might have changed following admixture. A concentration loss of 10 percent or greater was considered to be significant. This loss in potency has been advocated as a standard for the limits of loss of potency for a drug (75). Spectra were



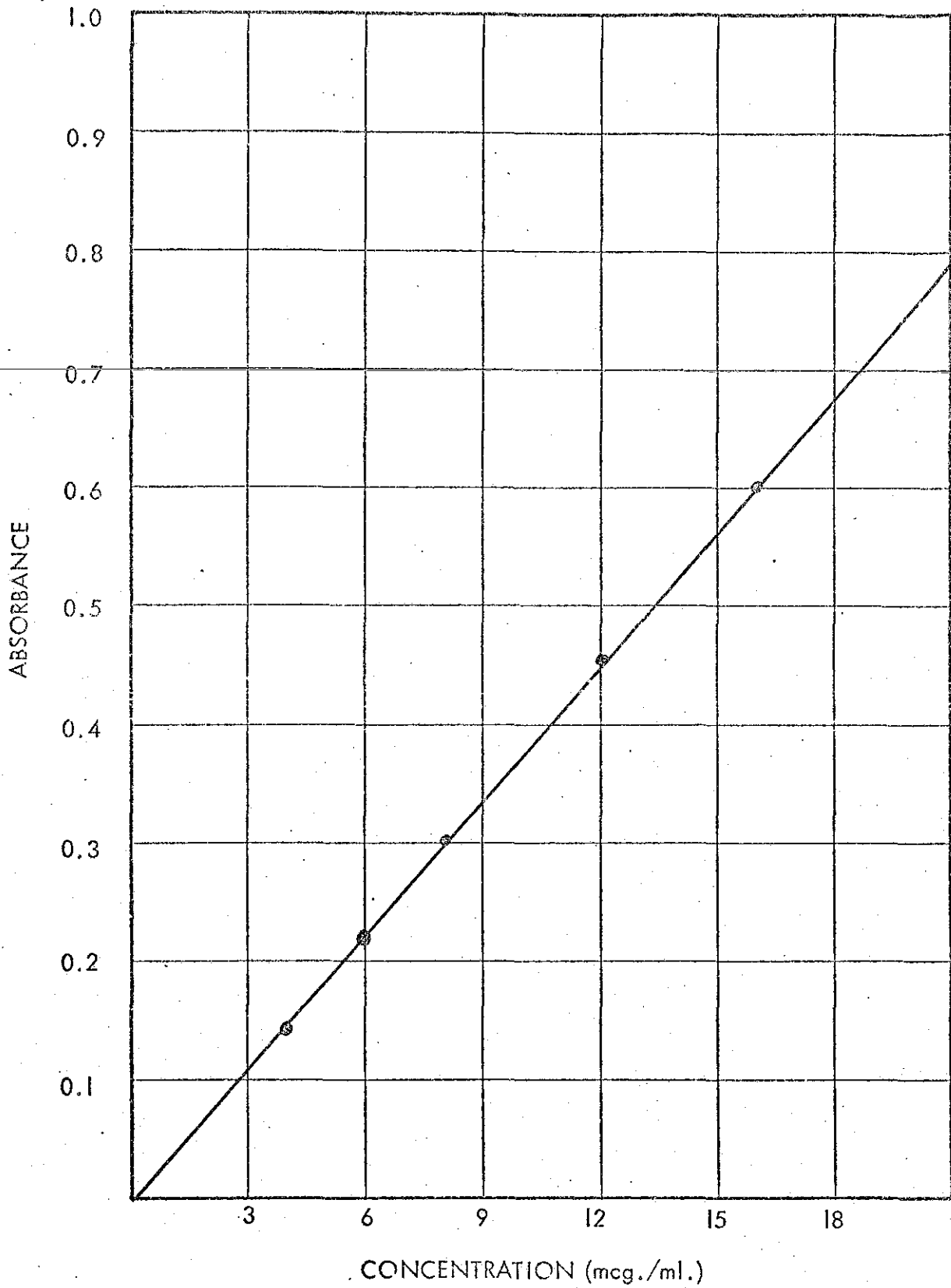
Graph 1. Beer plot for Prednisolone Sodium Phosphate
(λ_{\max} 257 m μ) in 5 percent Dextrose Injection.



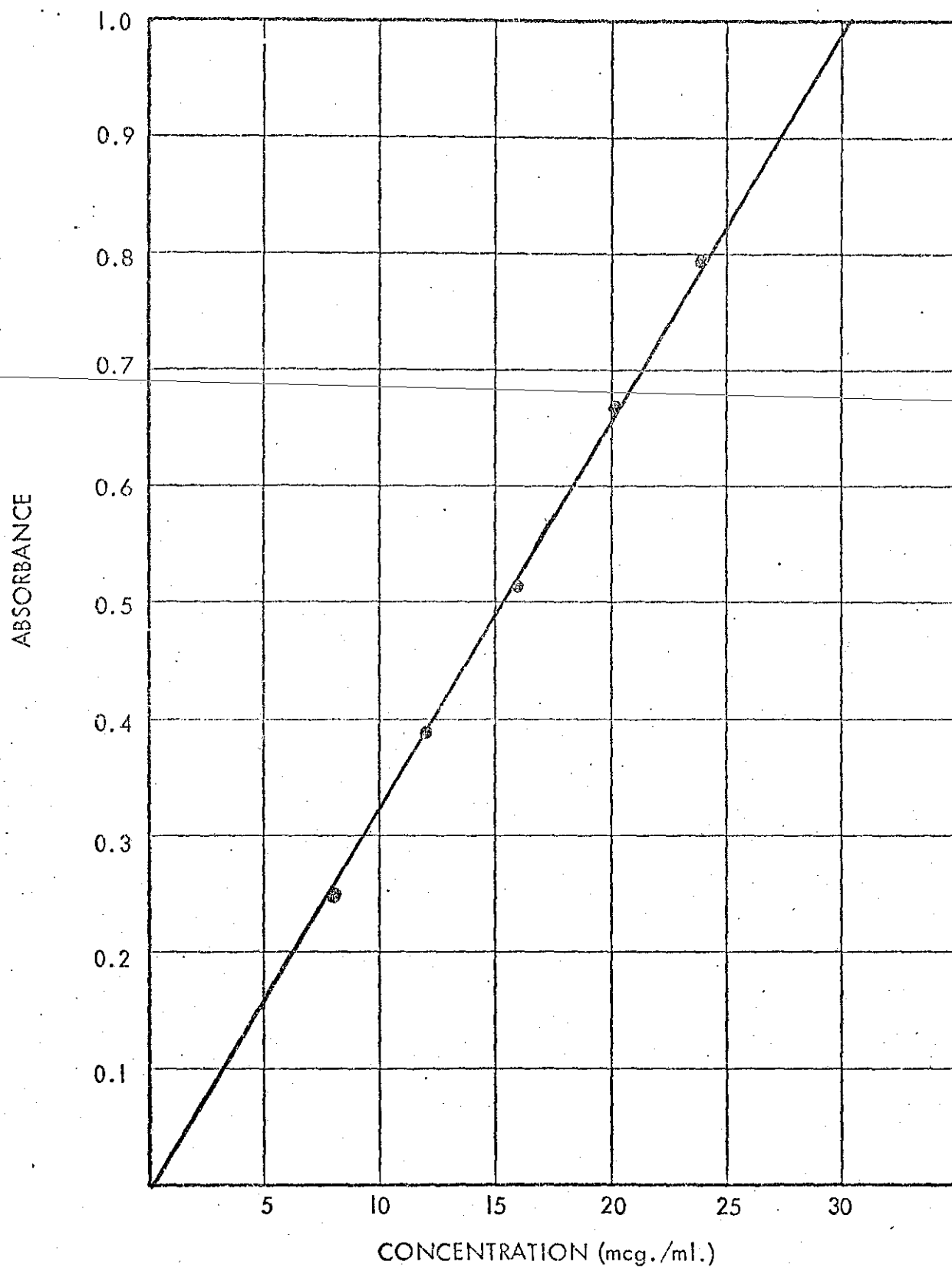
Graph 2. Beer plot for 5-Fluorouracil (λ_{\max} 265.5 m μ) in 5 percent Dextrose Injection.



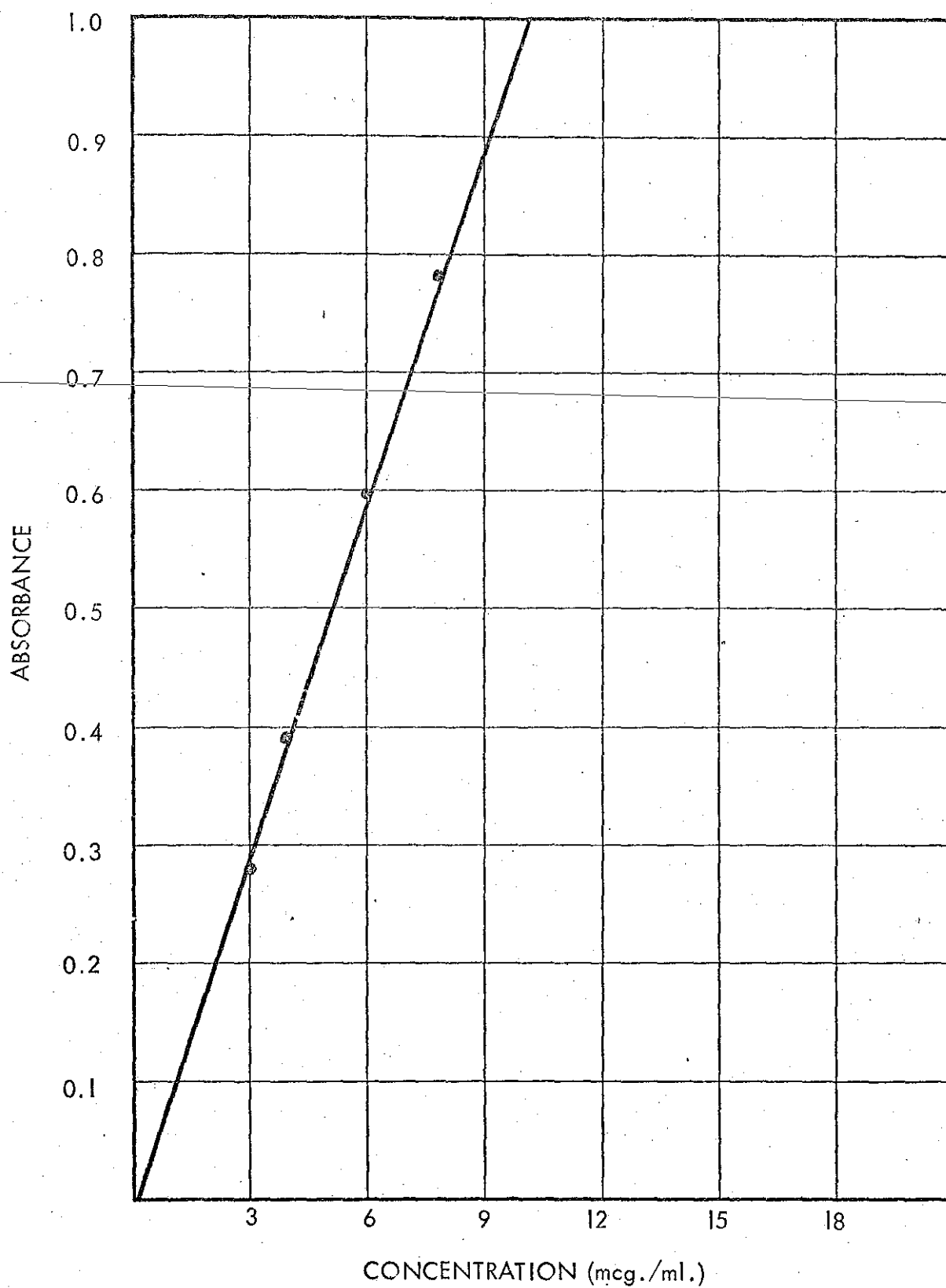
Graph 3. Beer plot for Methotrexate Sodium (λ_{\max} 302 m μ) in 5 percent Dextrose Injection.



Graph 4. Beer plot for Cytarabine (λ_{\max} 272 m μ) in 5 percent Dextrose Injection.



Graph 5. Beer plot for Sodium Cephalothin (λ_{\max} 236 m μ) in 5 percent Dextrose Injection.



Graph 6. Beer plot for Vincristine Sulfate (λ_{\max} 256.5 m μ) in 5 percent Dextrose Injection.

obtained at 1, 4, and 8 hour intervals to determine whether time would have an influence on the compatibility and stability of the drugs in admixture. Table III summarizes the results of the study using the spectrophotometric analysis technique.

ADMIXTURE #1 PREDNISOLONE SODIUM PHOSPHATE--5-FLUOROURACIL

Prednisolone sodium phosphate and 5-fluorouracil were added to 5 percent Dextrose Injection to yield concentrations of 200 mcg./ml. and 250 mcg./ml. respectively. These concentrations were considered to be within therapeutic range, calculated on the basis of a 70 Kg. person receiving the drugs over a 24 hour period in three divided doses, each of which was to be delivered in 500 ml. of intravenous fluid. The basis for this decision is summarized as follows:

	Prednisolone Sodium Phosphate	5-Fluorouracil
Usual Dose:	10-400 mg./day ^a	5-15-mg./Kg./day ^b
Dose for 70 Kg.	10-400 mg./day	350-1050 mg./day
Dose for 8 Hrs.	3.3-133 mg.	117-350 mg.
Therapeutic Conc.	200 mcg./ml.	250 mcg./ml.

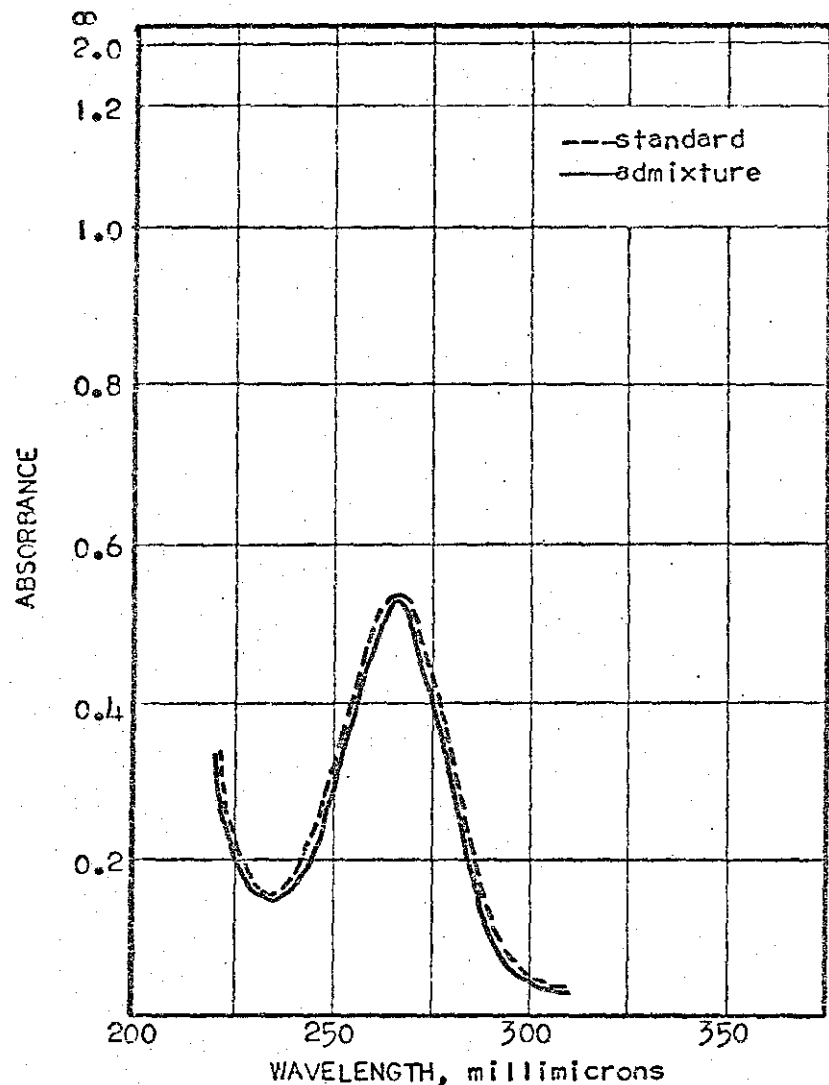
Since both drugs were available from the manufacturer in solution form, neither required reconstitution.

a - Product Information, Merck Sharp & Dohme, Division of Merck & Co., Inc., West Point, Pa.

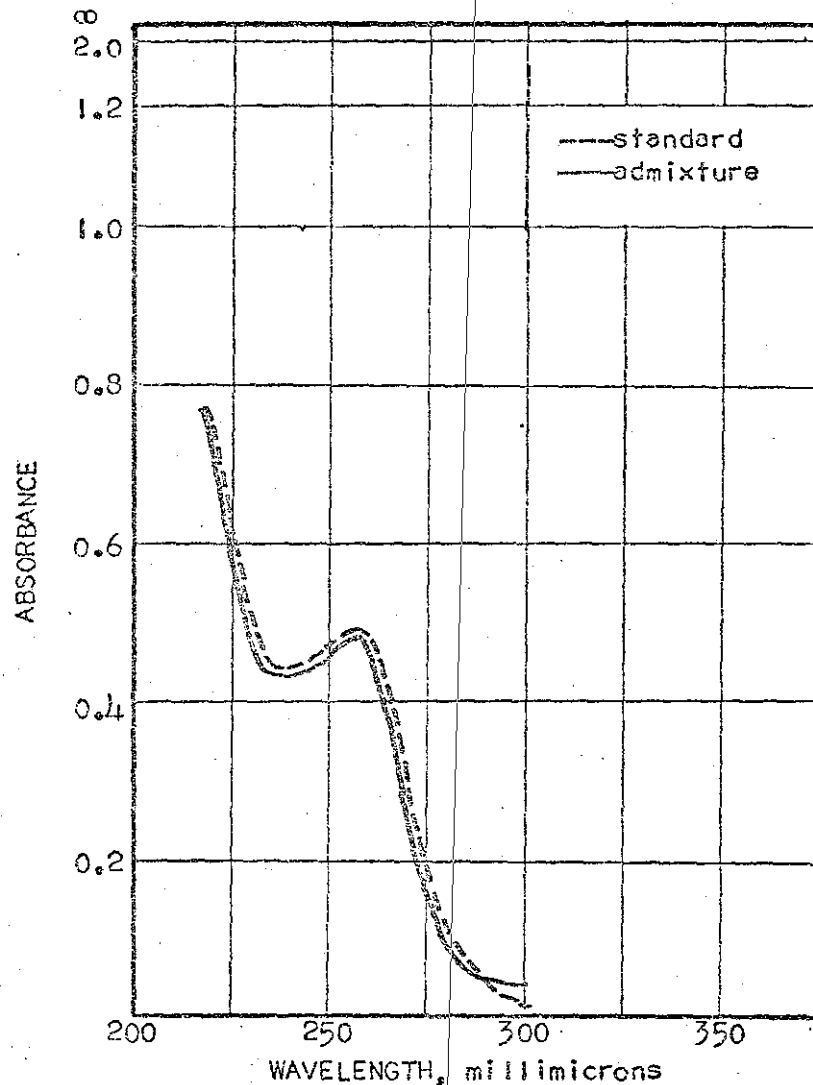
b - Product Information, Roche Laboratories, Division of Hoffmann La-Roche, Nutley, N. J.

In order to obtain an absorption spectrum for each of the drugs, it was necessary to dilute the therapeutic concentrations for scanning. The final dilutions resulted in a concentration of 8 mcg./ml. for prednisolone sodium phosphate and 10 mcg./ml. for 5-fluorouracil. Aliquot dilutions were done for each reading at the 1, 4, and 8 hour intervals.

~~For each of the two drugs, the absorption spectrum~~
of the admixture was compared with the standard spectrum for the individual agents (Figures 1, 2, and 3). The absorption spectrum for each of the drugs did not appear to be significantly altered either in shape or in degree of absorbance.

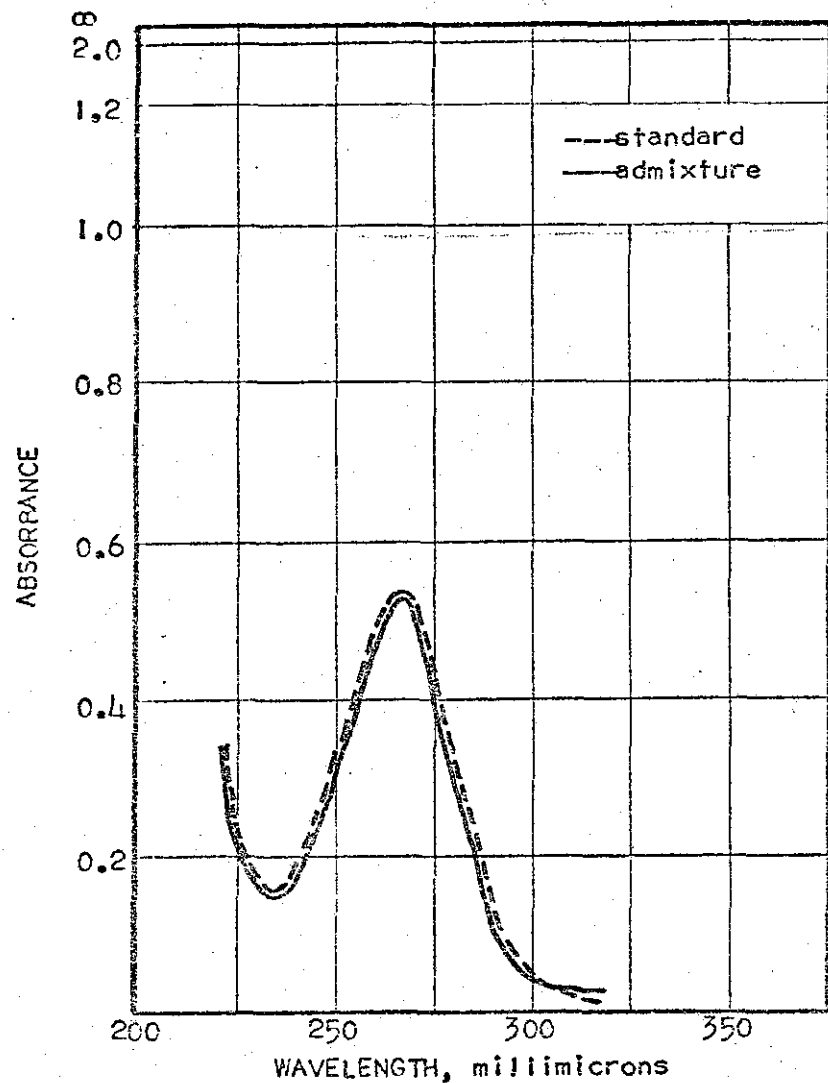


5-Fluorouracil 10 mcg./ml.
 Ref: Prednisolone Sodium Phosphate
 8 mcg./ml.

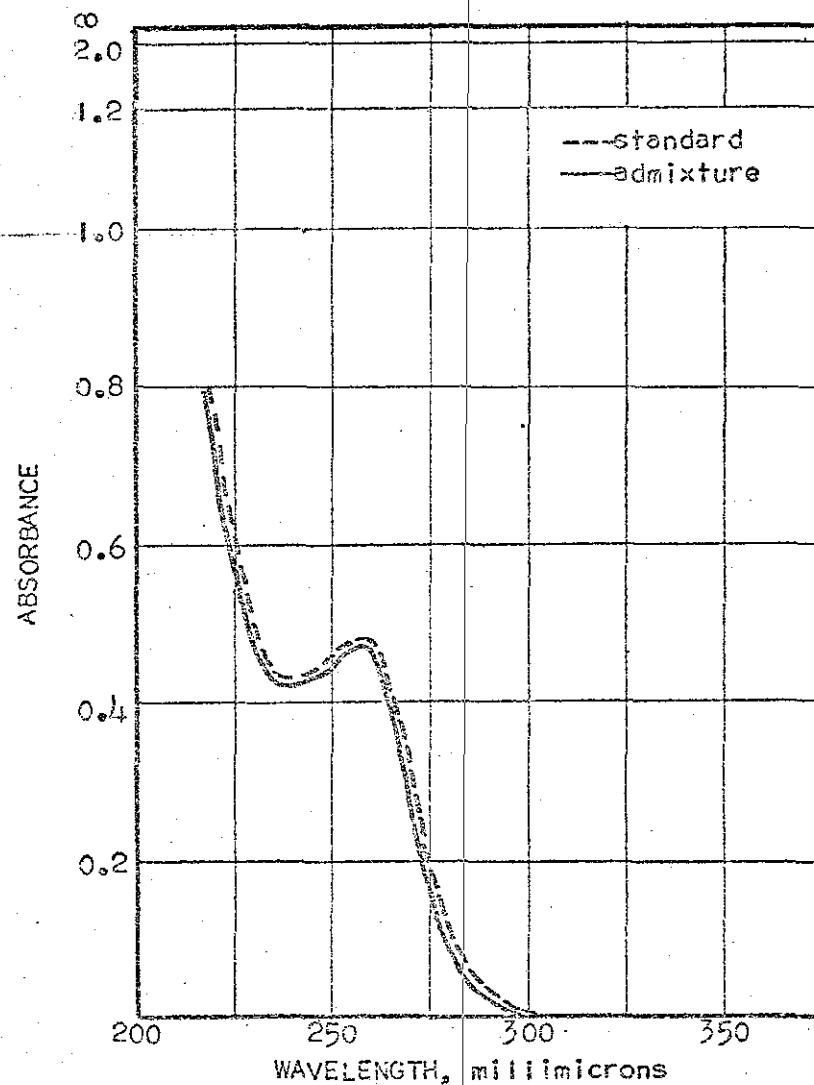


Prednisolone Sodium Phosphate 8 mcg./ml.
 Ref: 5-Fluorouracil 10 mcg./ml.

Figure 1. U.V. Spectrum of 5-Fluorouracil (λ_{\max} 265.5 mμ) with Prednisolone Sodium Phosphate (λ_{\max} 257 mμ) in 5 percent Dextrose Injection at one hour.

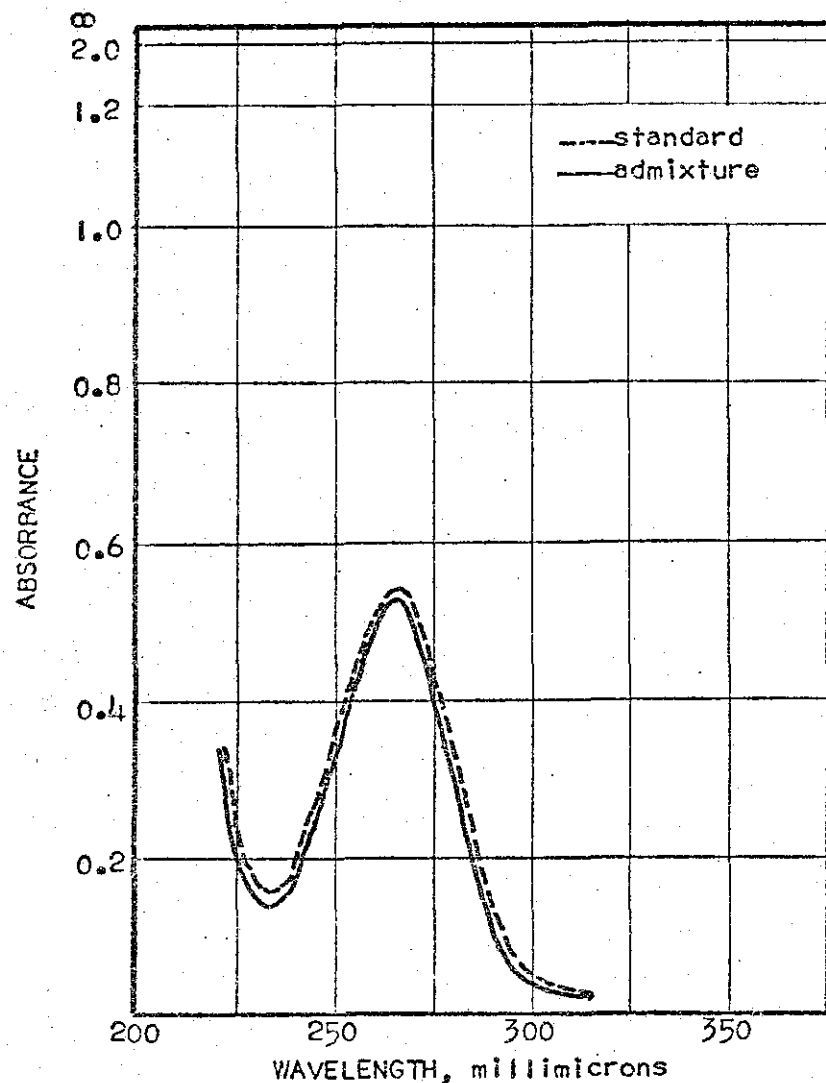


5-Fluorouracil 10 mcg./ml.
 Ref: Prednisolone Sodium Phosphate
 8 mcg./ml.

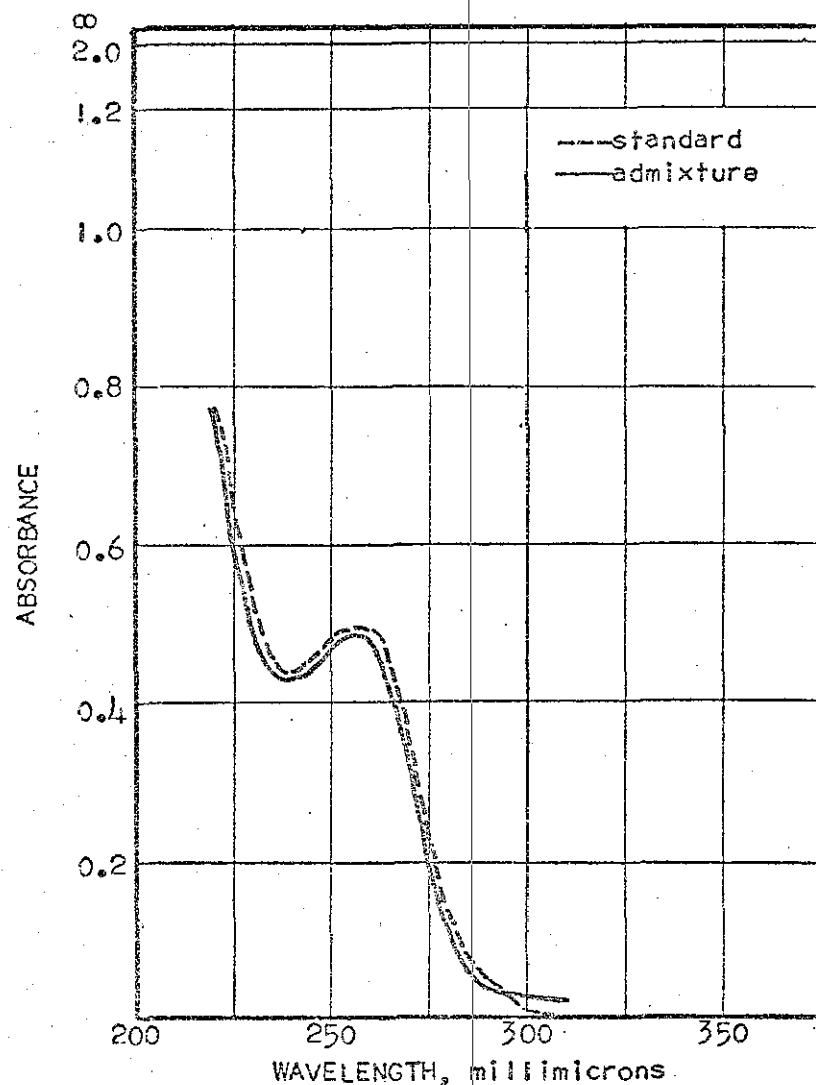


Prednisolone Sodium Phosphate 8 mcg./ml.
 Ref: 5-Fluorouracil 10 mcg./ml.

Figure 2. U.V. Spectrum of 5-Fluorouracil (λ_{max} 265.5 mμ) with Prednisolone Sodium Phosphate (λ_{max} 257 mμ) in 5 percent Dextrose Injection at four hours.



5-Fluorouracil 10 mcg./ml.
 Ref: Prednisolone Sodium Phosphate
 8 mcg./ml.



Prednisolone Sodium Phosphate 8 mcg./ml.
 Ref: 5-Fluorouracil 10 mcg./ml.

Figure 3. U.V. Spectrum of 5-Fluorouracil (λ_{max} 265.5 m μ) with Prednisolone Sodium Phosphate (λ_{max} 257 m μ) in 5 percent Dextrose Injection at eight hours.

ADMIXTURE #2 5-FLUOROURACIL--METHOTREXATE SODIUM

In the following admixture, 5-fluorouracil was added to methotrexate sodium. The concentration used for 5-fluorouracil was 250 mcg./ml. The concentration used for methotrexate sodium (200 mcg./ml.) was calculated on the following basis for a person receiving the drug over a 24 hour period in three divided doses, each of which to be delivered in 500 ml. of intravenous fluid:

	Methotrexate Sodium
Usual Dose Recommended	2 mg./Kg.--4 mg./Kg. ^a
Dose for 70 Kg. Adult	140 mg./day-280 mg./day
Dose for 8 Hr. Period	47 mg.--93 mg.
Therapeutic Conc. Used	200 mcg./ml.

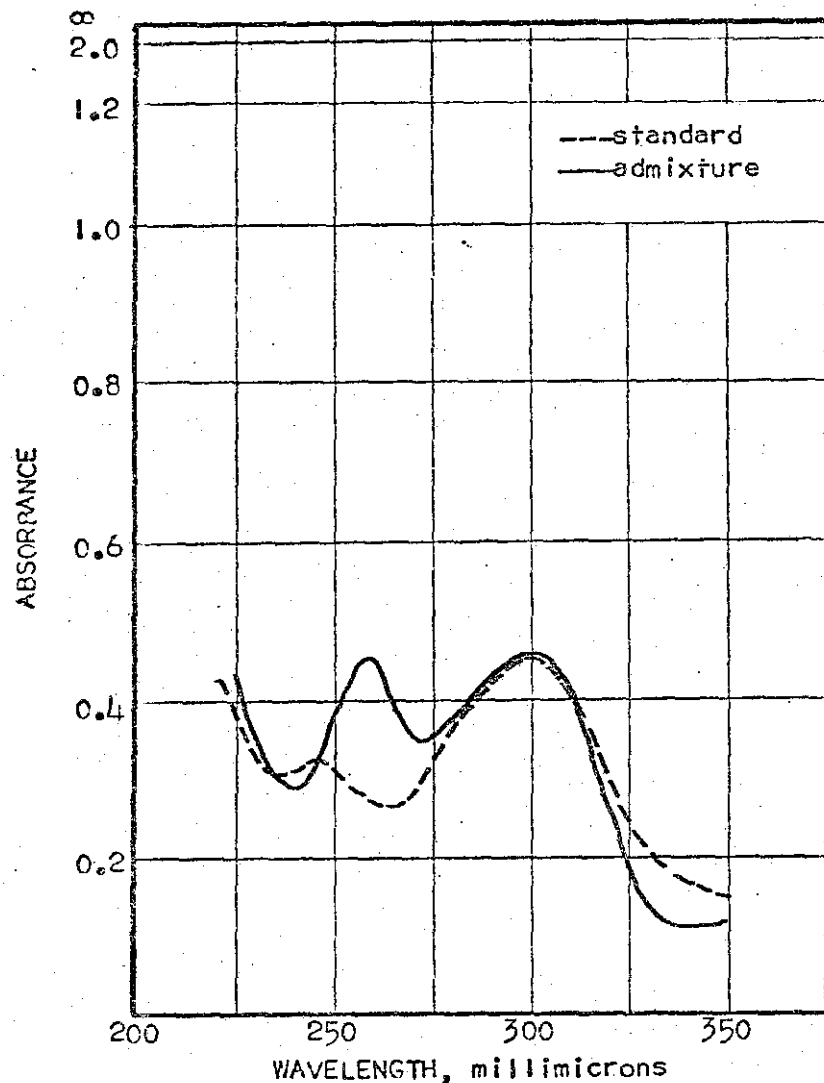
Commercially available methotrexate sodium is supplied in an aqueous solution form, therefore, no preliminary reconstitution of the product was needed.

The concentration of each drug in the admixture resembled a therapeutic quantity such as might be used in the clinical environment. Because these therapeutic concentrations were too great for spectrophotometric analysis, further dilution was necessary. At each test period indicated, appropriate dilutions were done so that the drugs could be scanned. The final concentrations of the dilutions used to obtain the spectrum of each drug in the admixture were for methotrexate sodium 8 mcg./ml. and

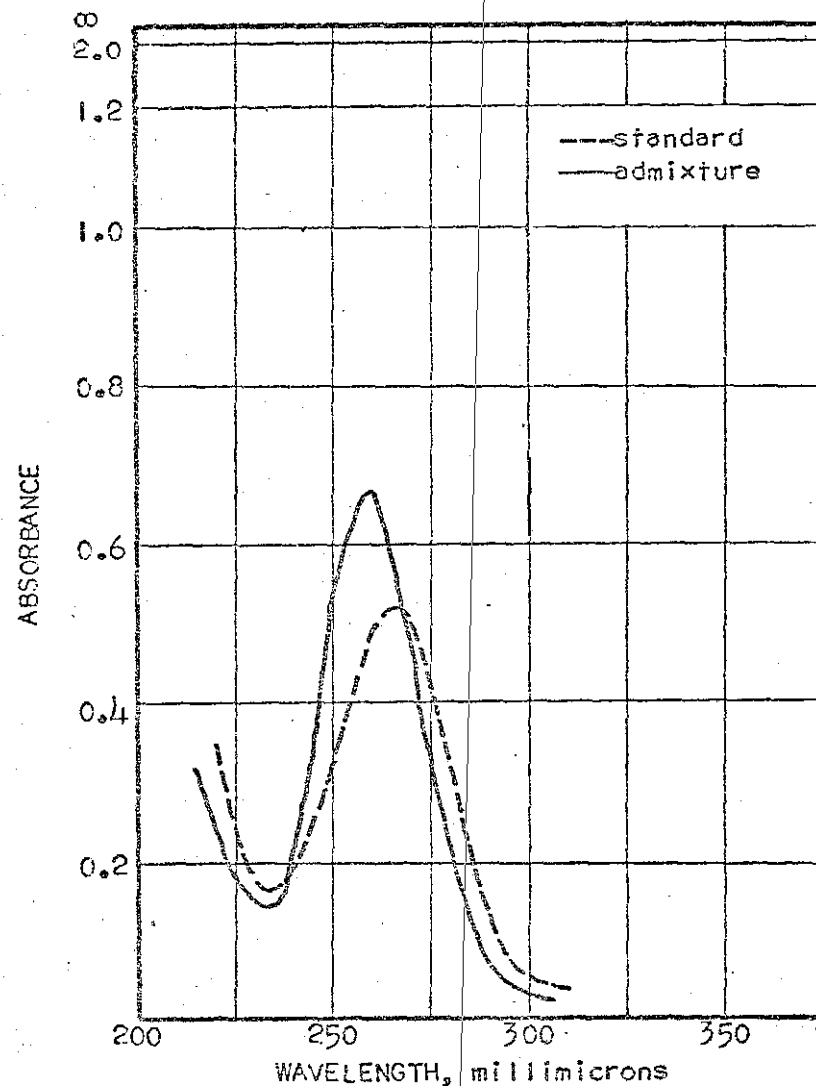
a - Product Information, Lederle Laboratories Division,
American Cyanamid Company, Pearl River, N. Y.

for 5-fluorouracil 10 mcg./ml.

In studying the resulting spectra by comparison to the reference or standard spectra and the Beer plots for each of the drugs, it was quite apparent that alterations in shape and magnitude of the absorption spectra had definitely occurred. From the onset of this study throughout the eight hour period, significant constant structural changes in the spectrum of methotrexate sodium were noticed, with the establishment of a new peak. 5-Fluorouracil, on the other hand, yielded a slight shift in its λ_{max} and a significant increase in absorbance. The results of this study would indicate the likely presence of a chemical interaction between these two drugs when in an admixture.

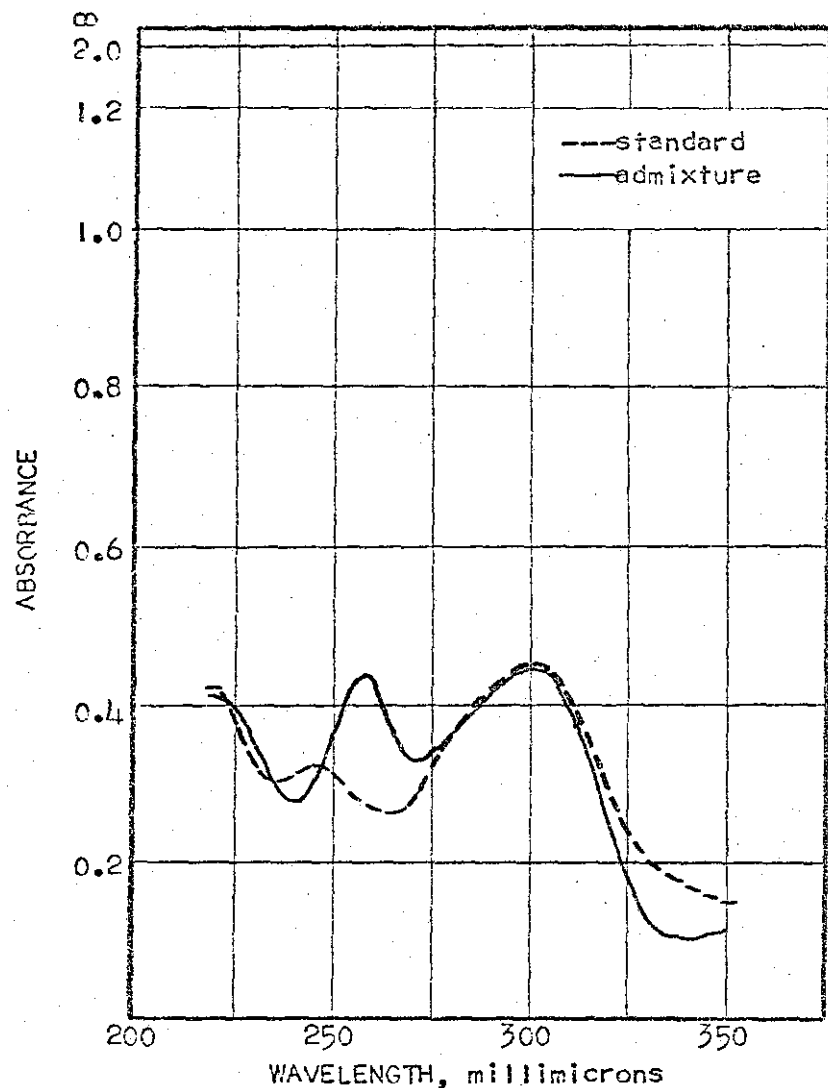


Methotrexate Sodium 8 mcg./ml.
Ref: 5-Fluorouracil 10 mcg./ml.

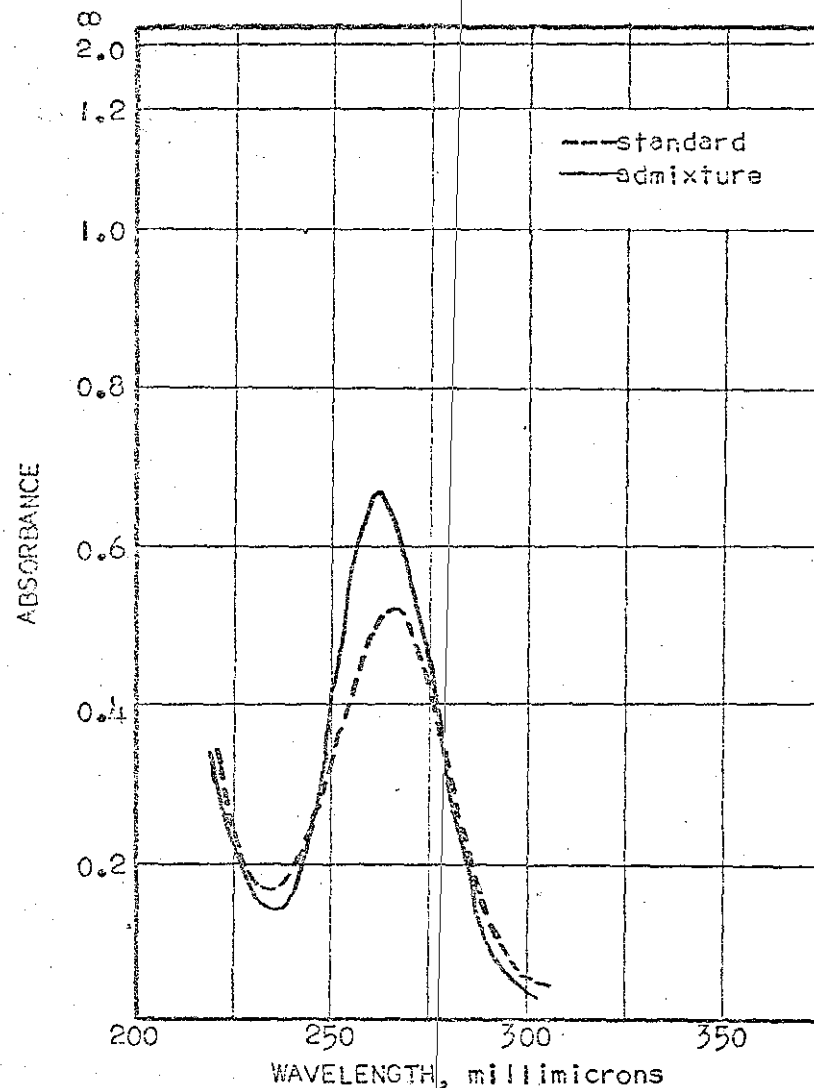


5-Fluorouracil 10 mcg./ml.
Ref: Methotrexate Sodium 8 mcg./ml.

Figure 4. U.V. Spectrum of Methotrexate Sodium (λ_{\max} 302 mμ) with 5-Fluorouracil (λ_{\max} 265.5 mμ) in 5 percent Dextrose Injection at one hour.

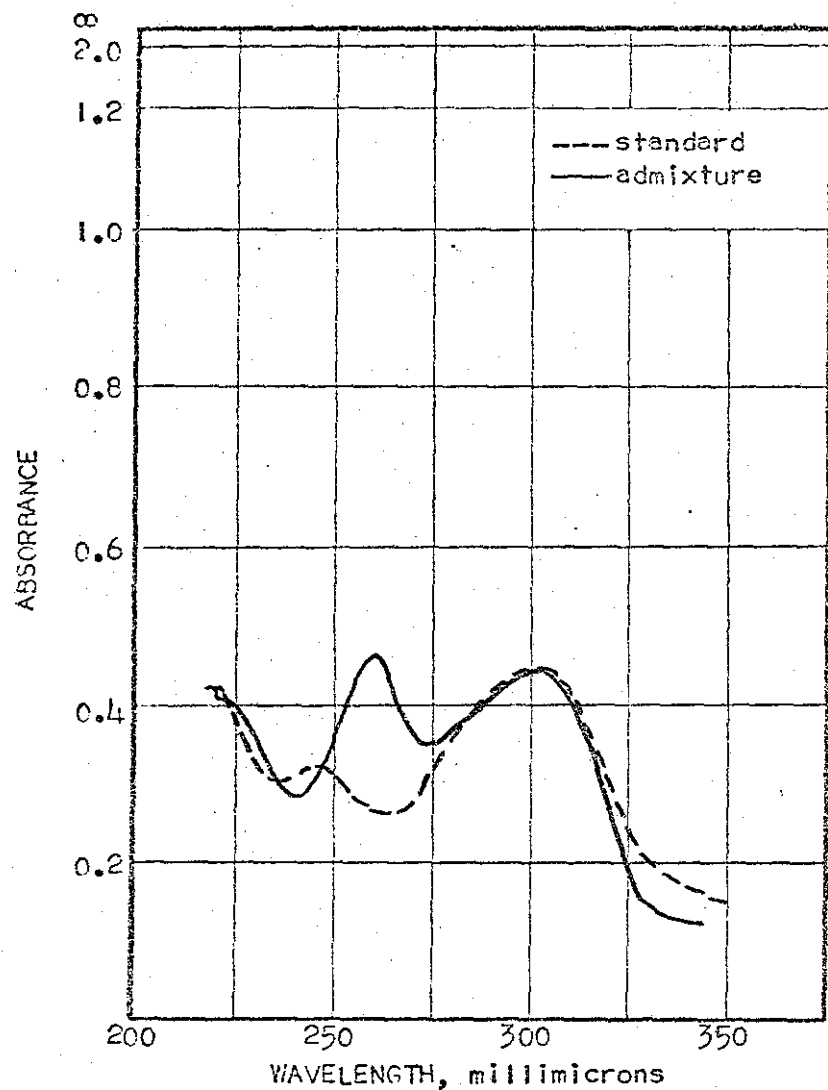


Methotrexate Sodium 8 mcg./ml.
Ref: 5-Fluorouracil 10 mcg./ml.

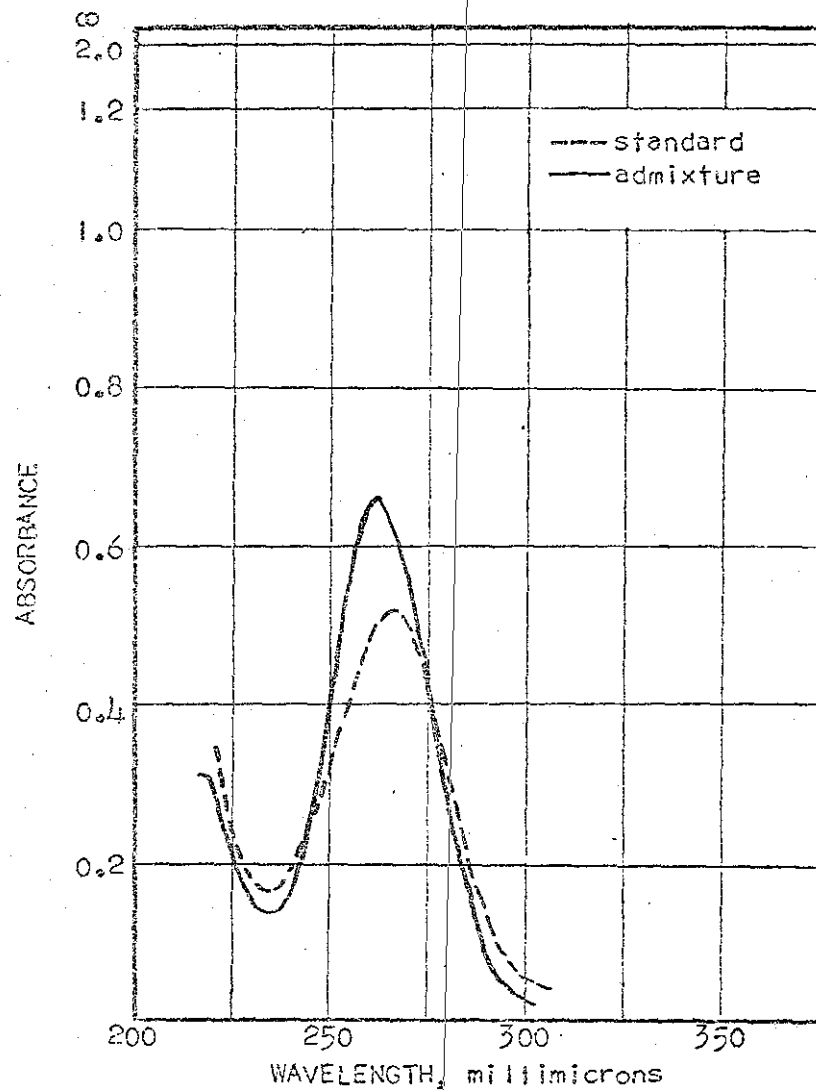


5-Fluorouracil 10 mcg./ml.
Ref: Methotrexate Sodium 8 mcg./ml.

Figure 5. U.V. Spectrum of Methotrexate Sodium (λ_{\max} 302 mμ) with 5-Fluorouracil (λ_{\max} 265.5 mμ) in 5 percent Dextrose Injection at four hours.



Methotrexate Sodium 8 mcg./ml.
Ref: 5-Fluorouracil 10 mcg./ml.



5-Fluorouracil 10 mcg./ml.
Ref: Methotrexate Sodium 8 mcg./ml.

Figure 6. U.V. Spectrum of Methotrexate Sodium (λ_{max} 302 mμ) with 5-Fluorouracil (λ_{max} 265.5 mμ) in 5 percent Dextrose Injection at eight hours.

ADMIXTURE #3 5-FLUOROURACIL-CYTARABINE

5-Fluorouracil, 250 mcg./ml. and cytarabine, 400 mcg./ml., were mixed in 5 percent Dextrose Injection. 5-Fluorouracil was added to the cytarabine at concentrations which might be used therapeutically. The concentrations of the 5-Fluorouracil was determined as in Admixture #1. The concentration of cytarabine was established according to a total daily dose divided into three portions, each delivered in 500 ml. of intravenous fluid:

	Cytarabine
Usual Dose Recommended	0.5-3 mg./Kg. ^a
Dose for 70 Kg. Person	3.5-210 mg./day
Dose for 8 Hr. Period	11.7-70 mg.
Therapeutic Conc.	400 mcg./ml.

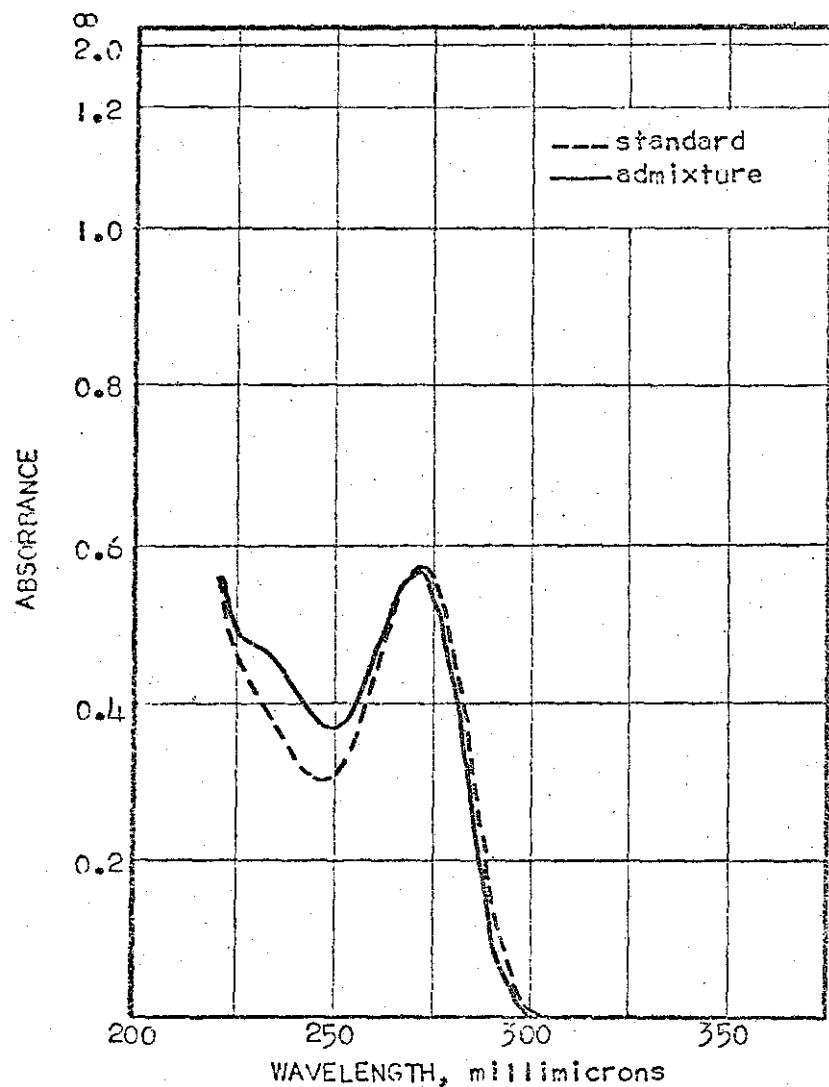
Since cytarabine is obtained in a lypholyzed state from the manufacturer, it was reconstituted according to the manufacturer's recommendations using the accompanying diluent.

In order to obtain an absorption spectrum for each of the drugs, it was necessary to dilute the therapeutic concentrations to lower concentrations to permit scanning within the limits of the spectrophotometer. Final dilutions were made to provide concentrations of 10 mcg./ml. for the 5-fluorouracil and 16 mcg./ml. for the cytarabine.

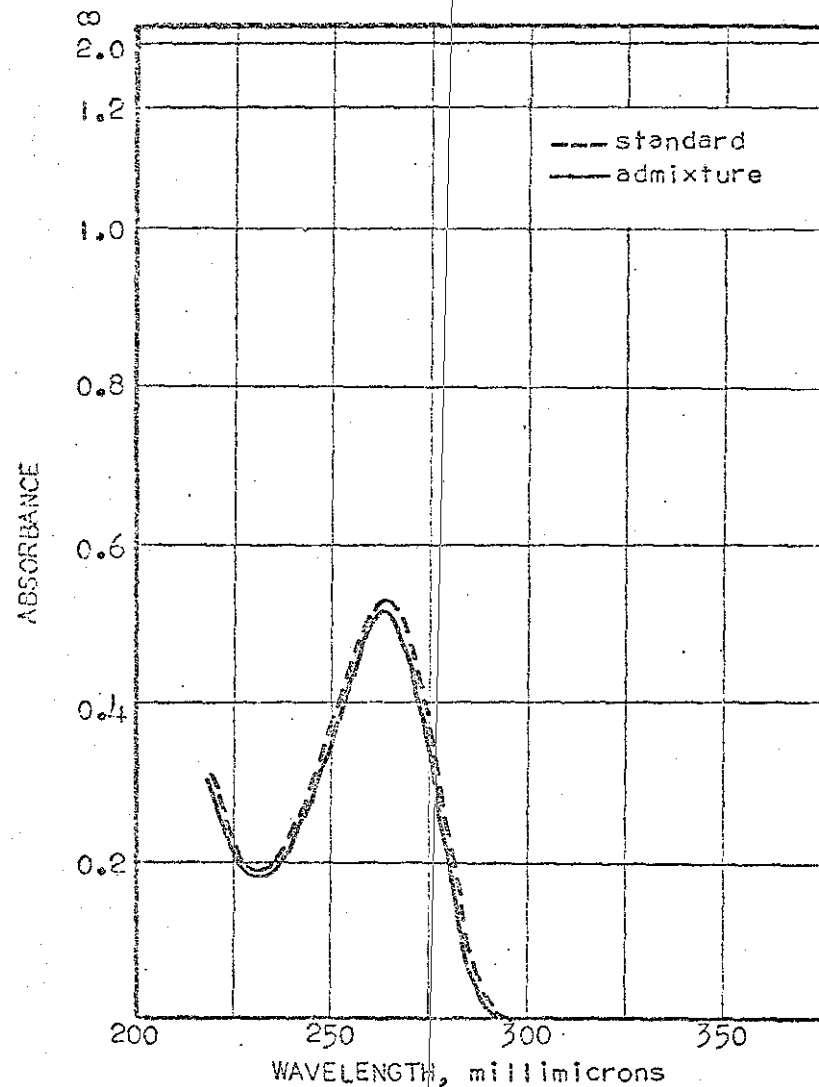
The absorption spectrum for 5-fluorouracil

a - Product Information, The Upjohn Company, Kalamazoo, Mich.

throughout the eight hour period was essentially unchanged. However, the ultraviolet absorption spectrum for the cytarabine was slightly changed by an alteration in the initial loop of the spectrum. There appeared to be no change in the main absorption peak or λ_{max} of the spectrum. Nevertheless, this might be an indication of a change in the chemical structure of the drug. (Figures 7, 8, 9)

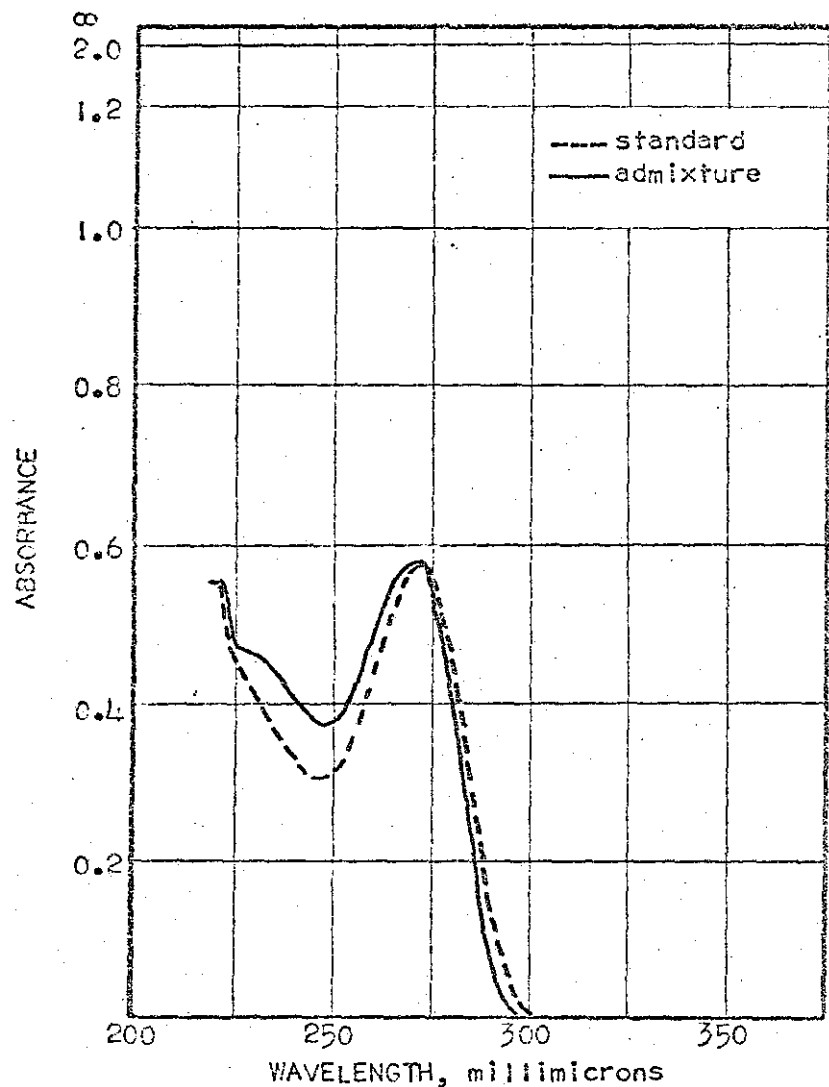


Cytarabine 16 mcg./ml.
Ref: 5-Fluorouracil 10 mcg./ml.

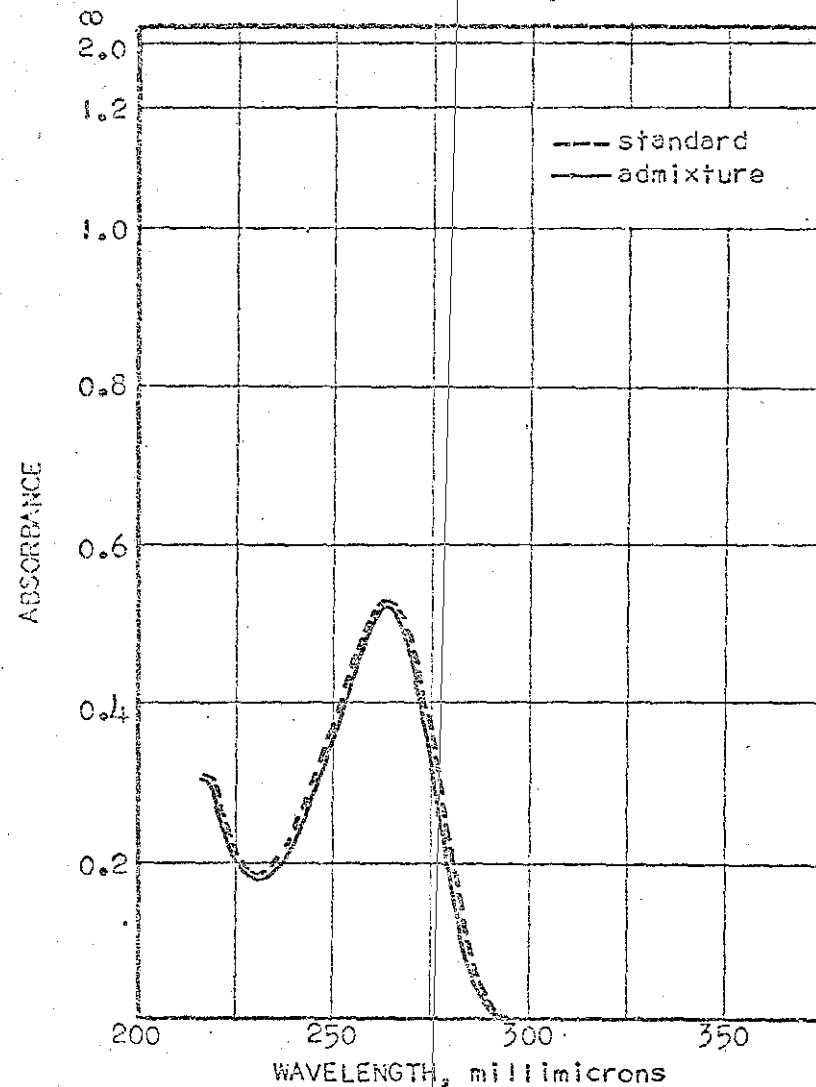


5-Fluorouracil 10 mcg./ml.
Ref: Cytarabine 16 mcg./ml.

Figure 7. U.V. Spectrum of Cytarabine (λ_{\max} 272 mμ) with 5-Fluorouracil (λ_{\max} 265.5 mμ) in 5 percent Dextrose Injection at one hour.

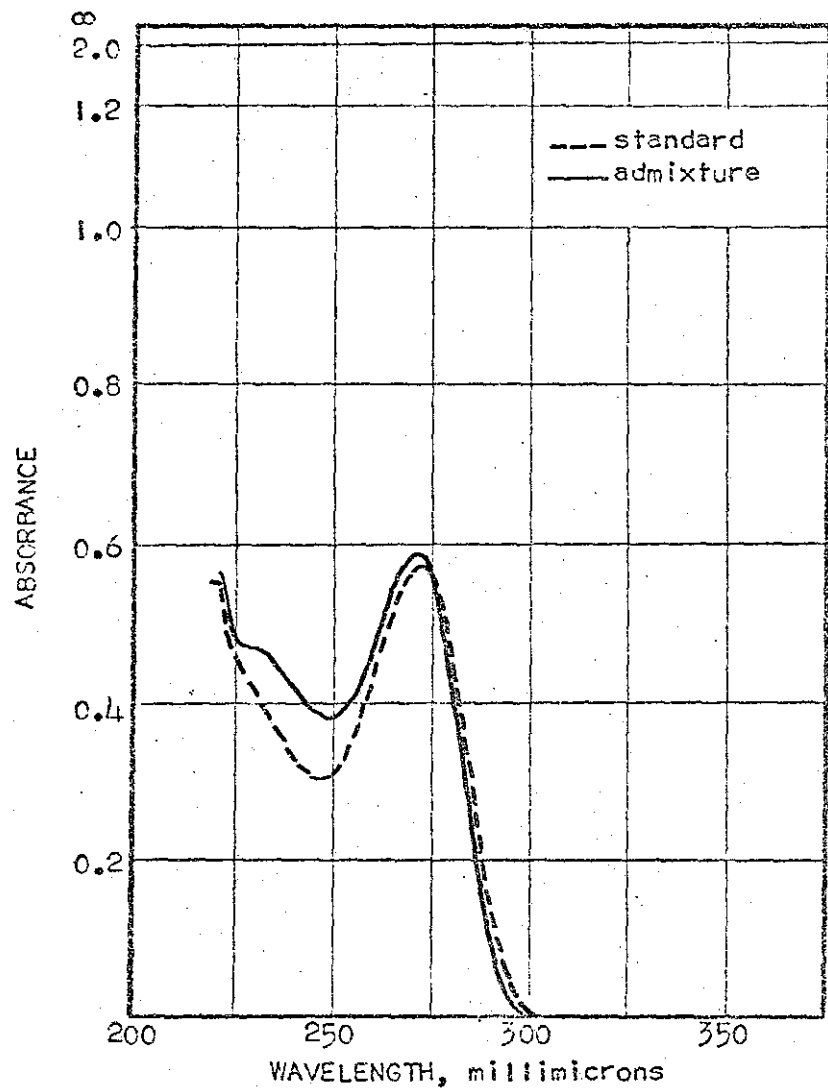


Cytarabine 16 mcg./ml.
Ref: 5-Fluorouracil 10 mcg./ml.

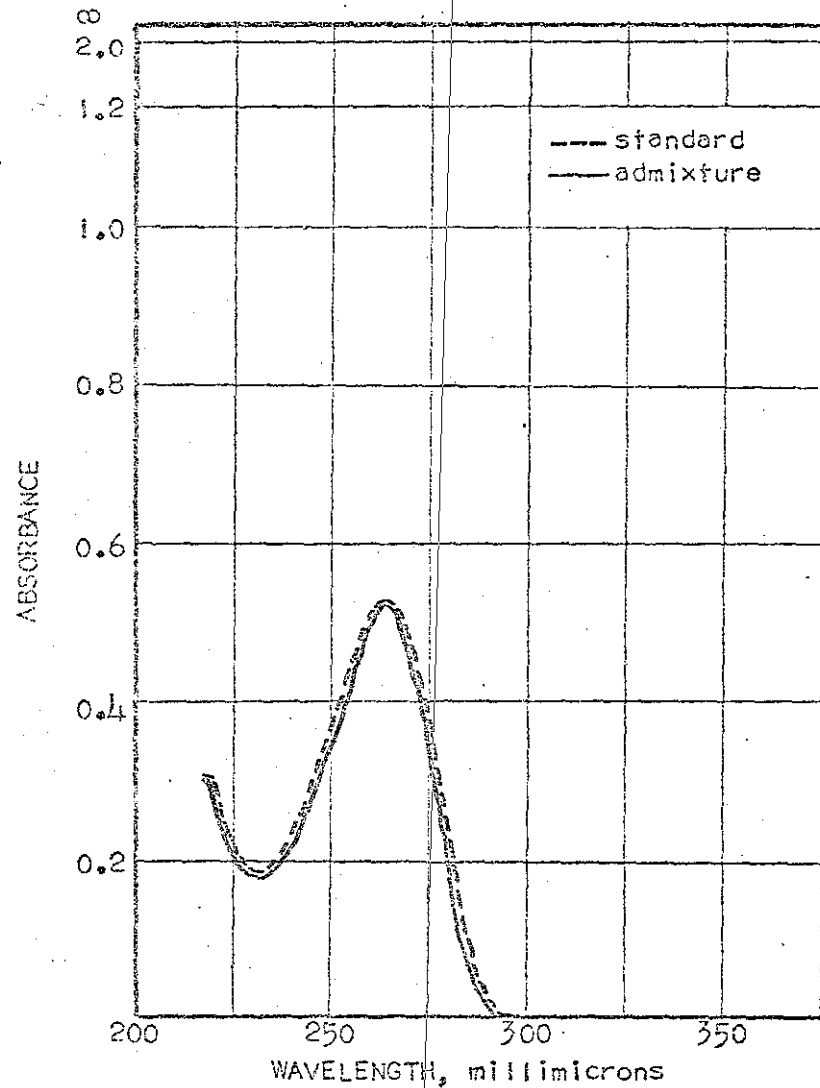


5-Fluorouracil 10 mcg./ml.
Ref: Cytarabine 16 mcg./ml.

Figure 8. U.V. Spectrum of Cytarabine (λ_{max} 272 mμ) with 5-Fluorouracil (λ_{max} 265.5 mμ) in 5 percent Dextrose Injection at four hours.



Cytarabine 16 mcg./ml.
Ref: 5-Fluorouracil 10 mcg./ml.



5-Fluorouracil 10 mcg./ml.
Ref: Cytarabine 16 mcg./ml.

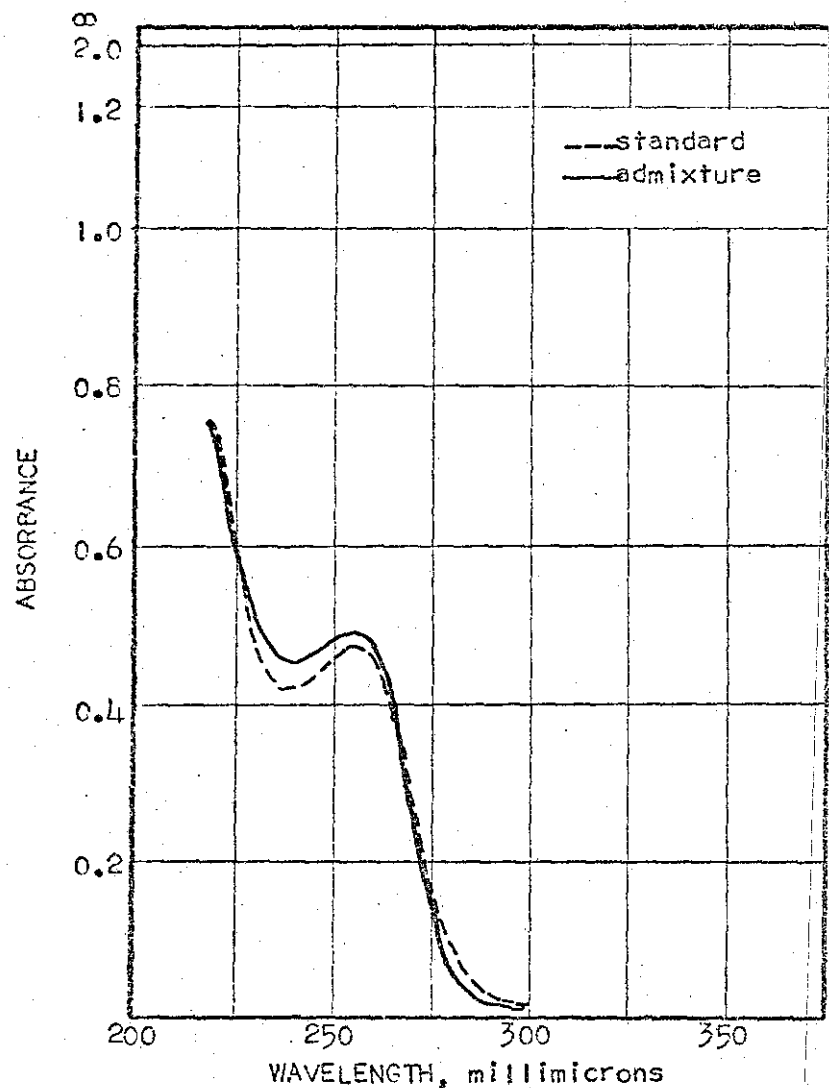
Figure 9. U.V. Spectrum of Cytarabine (λ_{\max} 272 mμ) with 5-Fluorouracil (λ_{\max} 265.5 mμ) in 5 percent Dextrose Injection at eight hours.

ADMIXTURE #4 PREDNISOLONE SODIUM PHOSPHATE--CYTARABINE

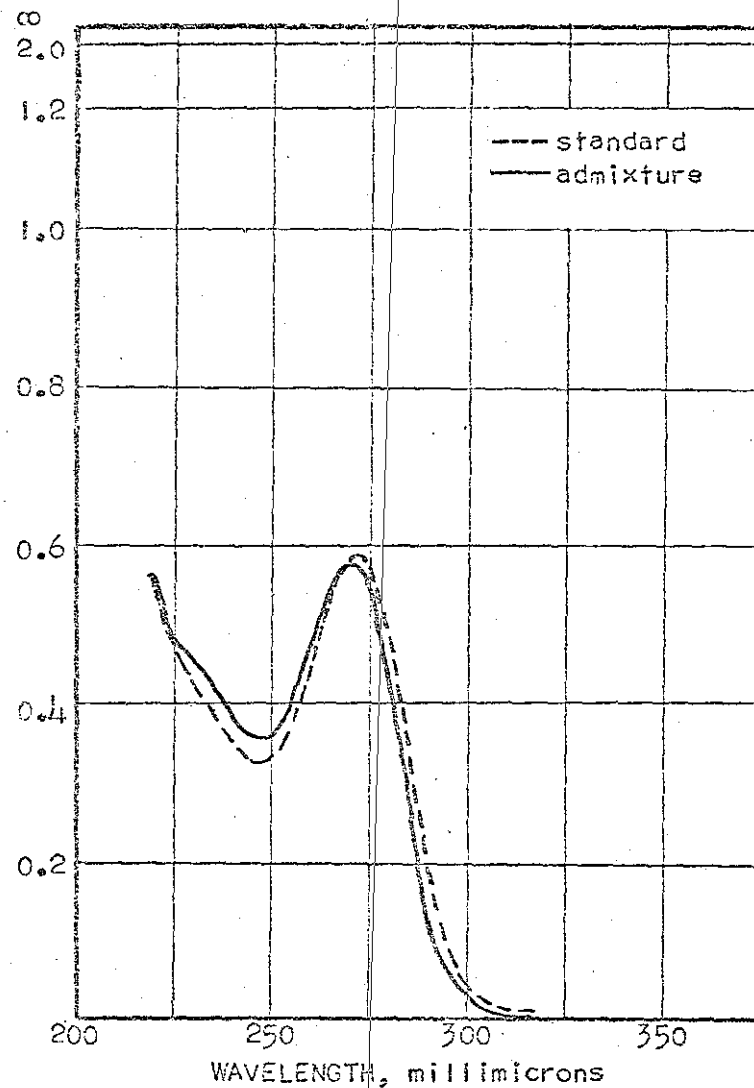
The figures for the following study include the spectra from the admixture of prednisolone sodium phosphate and cytarabine. The concentration used for the former drug was 200 mcg./ml. and for the latter was 400 mcg./ml. Both concentrations were considered to be within therapeutic range for an average adult. In this admixture, the ~~prednisolone sodium phosphate~~ was added to the cytarabine in the infusion fluid.

Because the solutions at therapeutic concentrations of these drugs were too dense optically to be scanned by the spectrophotometer, dilutions of the admixtures were necessary. The final dilutions resulted in concentrations of 8 mcg./ml. for the prednisolone sodium phosphate and of 16 mcg./ml. for the cytarabine. These dilutions were prepared for each readings at the 1, 4, and 8 hour intervals.

The absorbance for each drug was not significantly decreased, and neither spectrum was altered during the eight hour period. It would appear that this admixture is compatible and no chemical or physical incompatibility occurs when these two drugs are mixed together in 5 percent Dextrose Injection. (Figures 10, 11, 12)

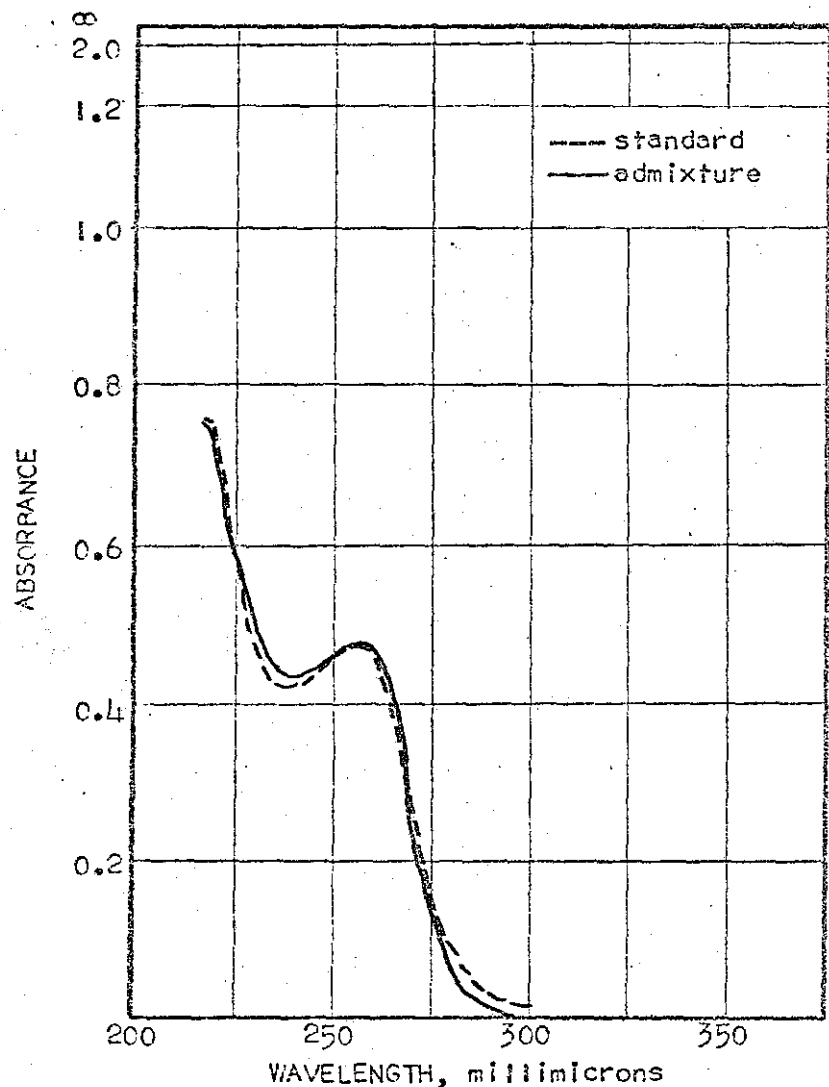


Prednisolone Sodium Phosphate 8 mcg./ml.
Ref: Cytarabine 16 mcg./ml.

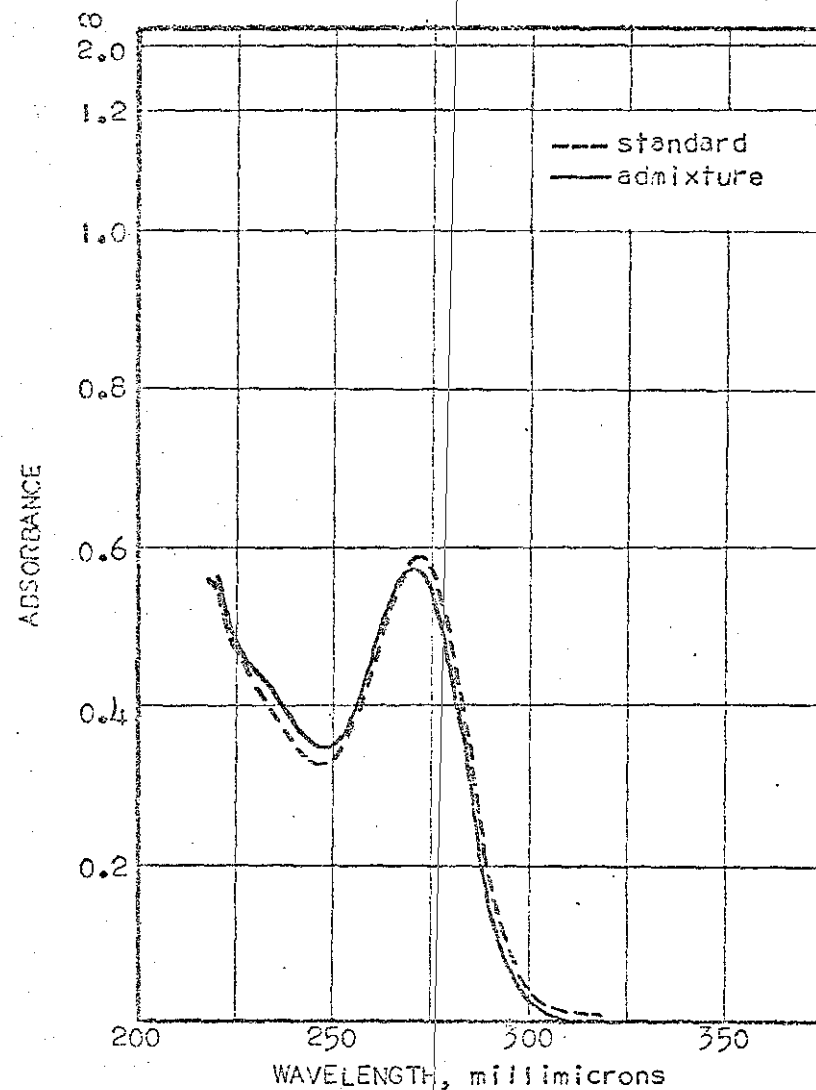


Cytarabine 16 mcg./ml.
Ref: Prednisolone Sodium Phosphate 8 mcg./ml.

Figure 10. U. S. Spectrum of Prednisolone Sodium Phosphate (λ_{max} 257 mμ) with Cytarabine (λ_{max} 272 mμ) in 5 percent Dextrose Injection at one hour.

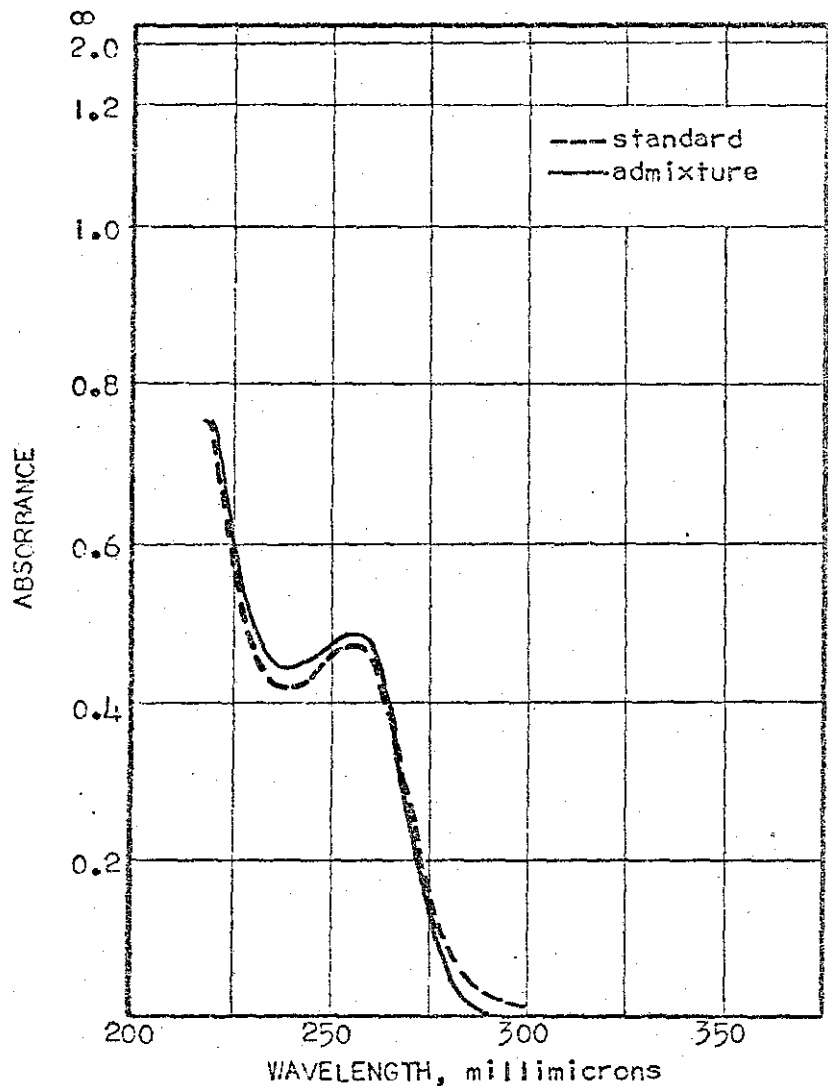


Prednisolone Sodium Phosphate 8 mcg./ml.
Ref: Cytarabine 16 mcg./ml.

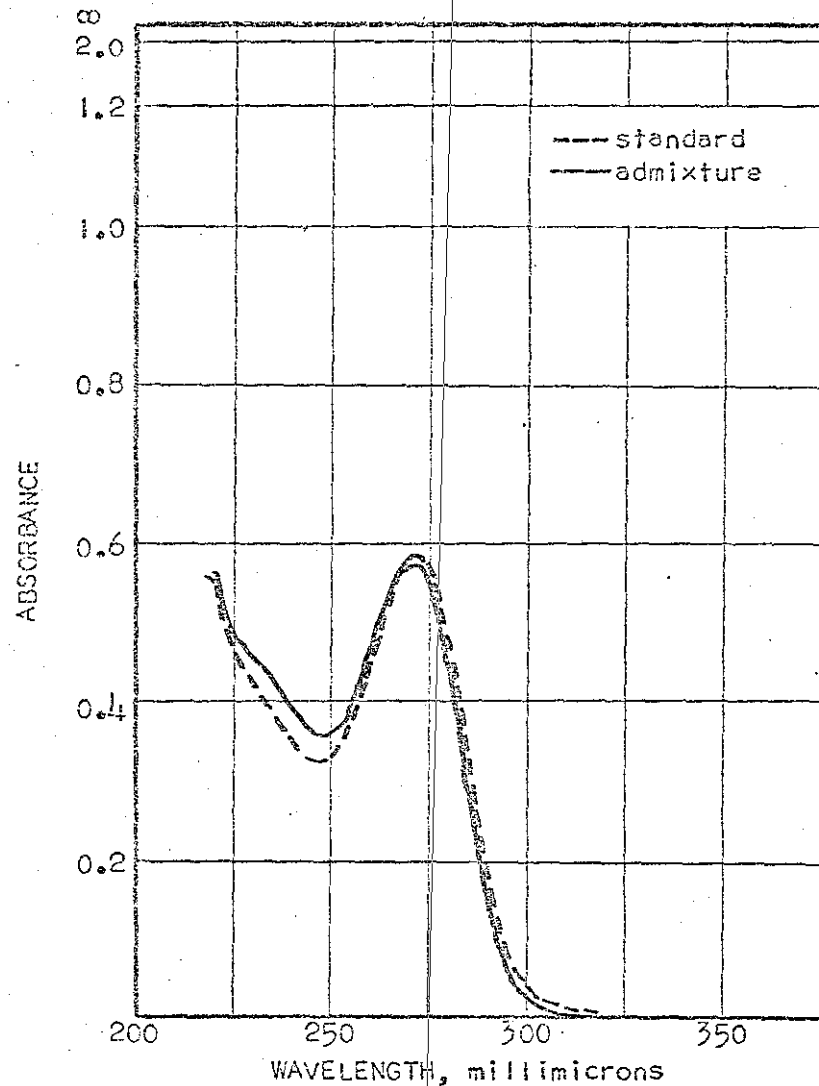


Cytarabine 16 mcg./ml.
Ref: Prednisolone Sodium Phosphate 8 mcg./ml.

Figure 11. U.V. Spectrum of Prednisolone Sodium Phosphate (λ_{\max} 257 mμ) with Cytarabine (λ_{\max} 272 mμ) in 5 percent Dextrose Injection at four hours.



Prednisolone Sodium Phosphate 8 mcg./ml.
Ref: Cytarabine 16 mcg./ml.



Cytarabine 16 mcg./ml.
Ref: Prednisolone Sodium Phosphate 8 mcg./ml.

Figure 12. U.V. Spectrum of Prednisolone Sodium Phosphate (λ_{max} 257 mμ) with Cytarabine (λ_{max} 272 mμ) in 5 percent Dextrose Injection at eight hours.

ADMIXTURE #5 5-FLUOROURACIL--SODIUM CEPHALOTHIN

In the following admixture, 5-fluorouracil, 500 mcg./ml. was added to sodium cephalothin, 1,000 mcg./ml. The large doses normally used for sodium cephalothin (from 500 mg. to 4 Gm. per I.V.) necessitated using a concentration of 5-fluorouracil double that used previously in this study. In order to dilute both drugs accurately, ~~with only one dilution and still~~ obtain a concentration for both drugs from which an absorption could be obtained, it was necessary to increase the concentration of the 5-fluorouracil to approximate the concentration used for the sodium cephalothin more closely.

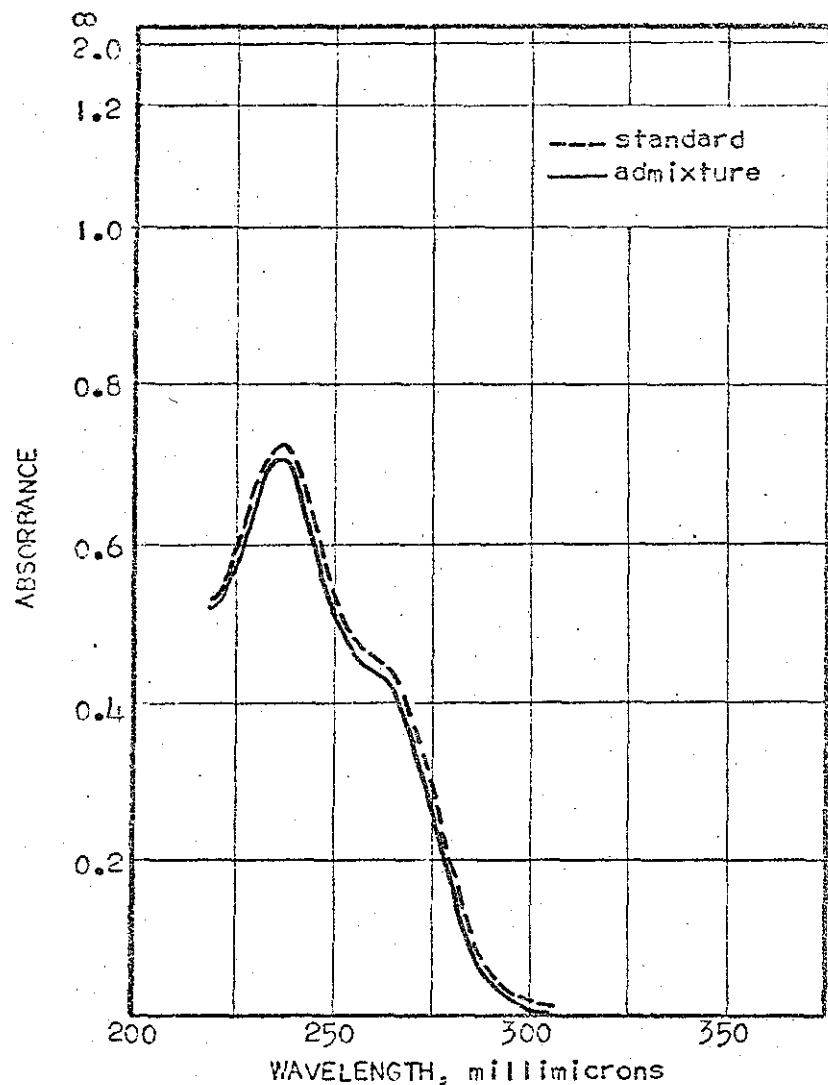
Since sodium cephalothin was available only in the dry powder form, it was reconstituted according to the manufacturer's recommendations using 10 ml. of Sterile Water for Injection as the diluent.

The therapeutic concentration used for the sodium cephalothin was decided upon arbitrarily using a lower convenient dose of 500 mg./500 ml. or 1,000 mcg./ml.

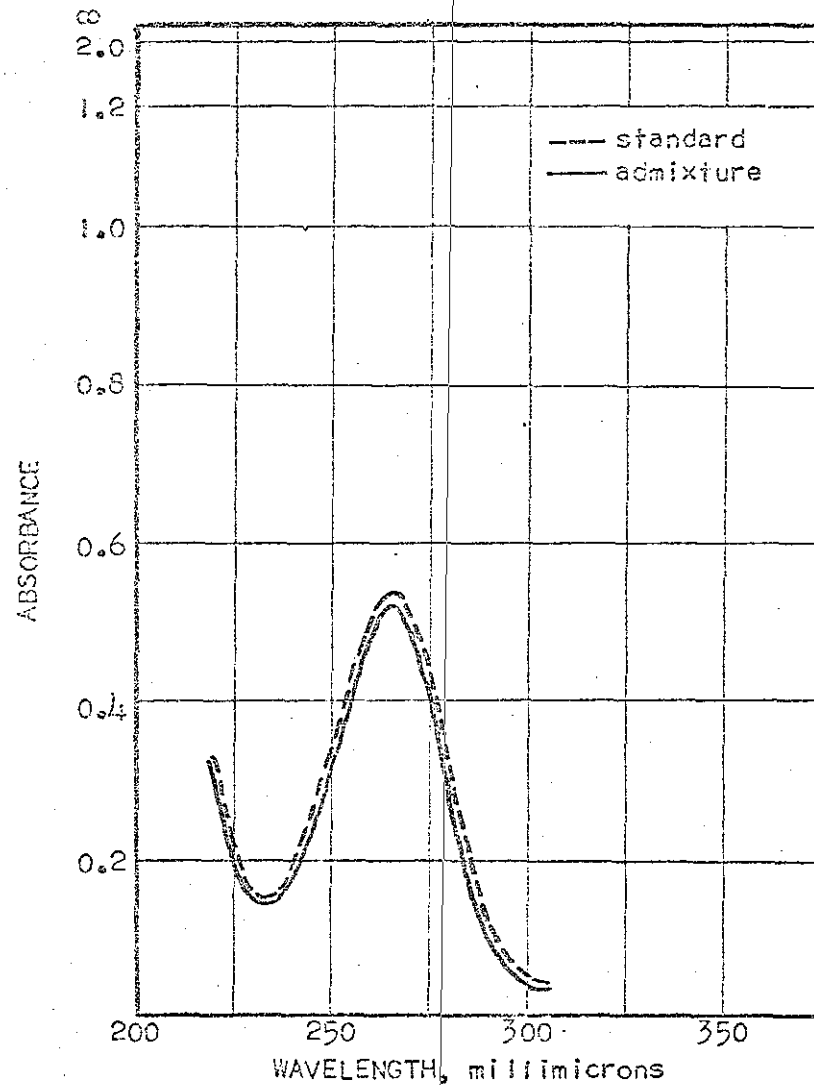
Aliquot portions of the therapeutic admixtures were used for the final dilutions that could be scanned by the spectrophotometer. The concentrations of the dilutions were for the 5-fluorouracil, 10 mcg./ml. and for the sodium cephalothin, 20 mcg./ml.

Admixture analysis revealed no significant decrease in the absorbance of either drug and no apparent alteration

in the spectra when compared to the reference spectra. There also were no visual signs of any incompatibility. It would appear that this admixture combination is physically and chemically compatible. (Figures 13, 14, 15)

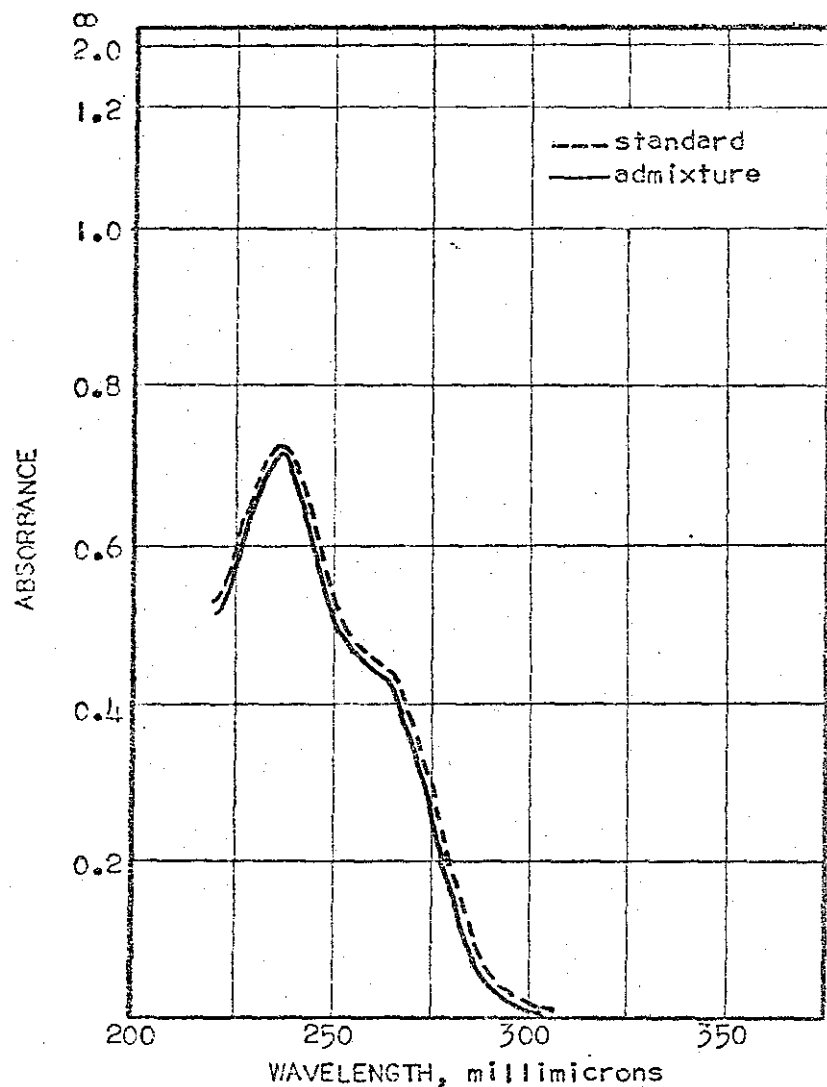


Sodium Cephalothin 20 mcg./ml.
Ref: 5-Fluorouracil 10 mcg./ml.

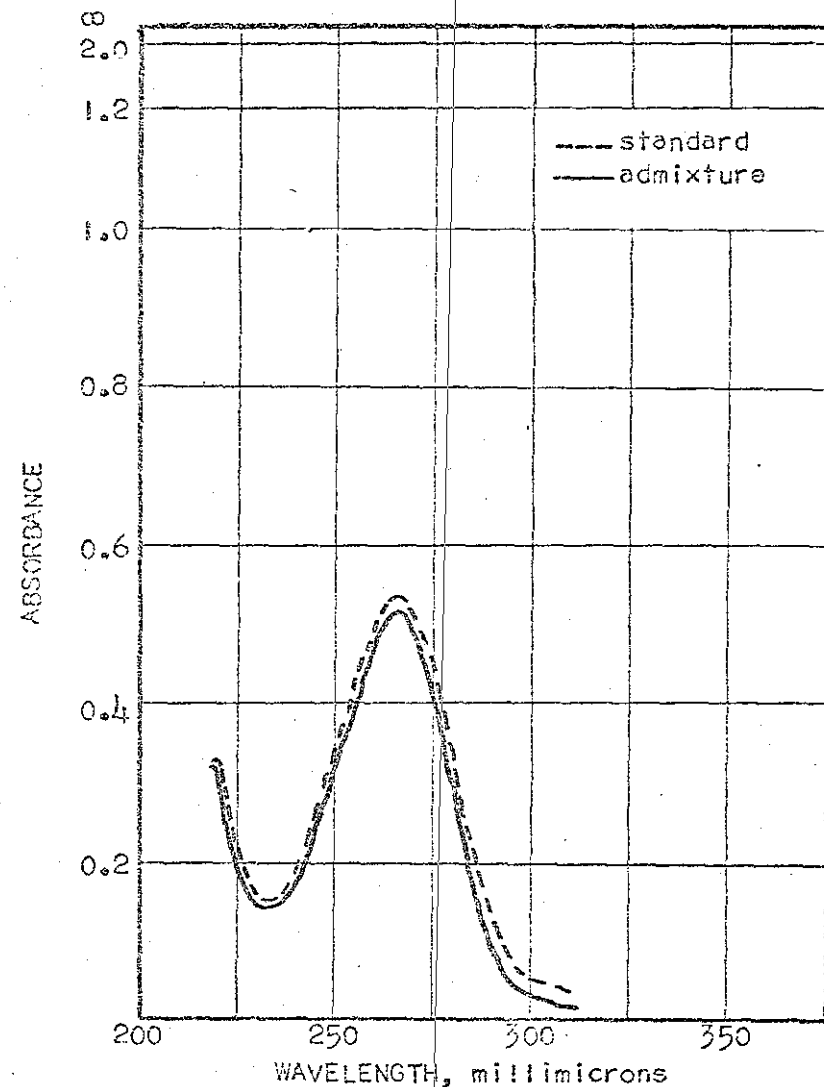


5-Fluorouracil 10 mcg./ml.
Ref: Sodium Cephalothin 20 mcg./ml.

Figure 13. U.V. Spectrum of Sodium Cephalothin (λ_{\max} 236 mμ) with 5-Fluorouracil (λ_{\max} 265.5 mμ) in 5 percent Dextrose Injection at one hour.

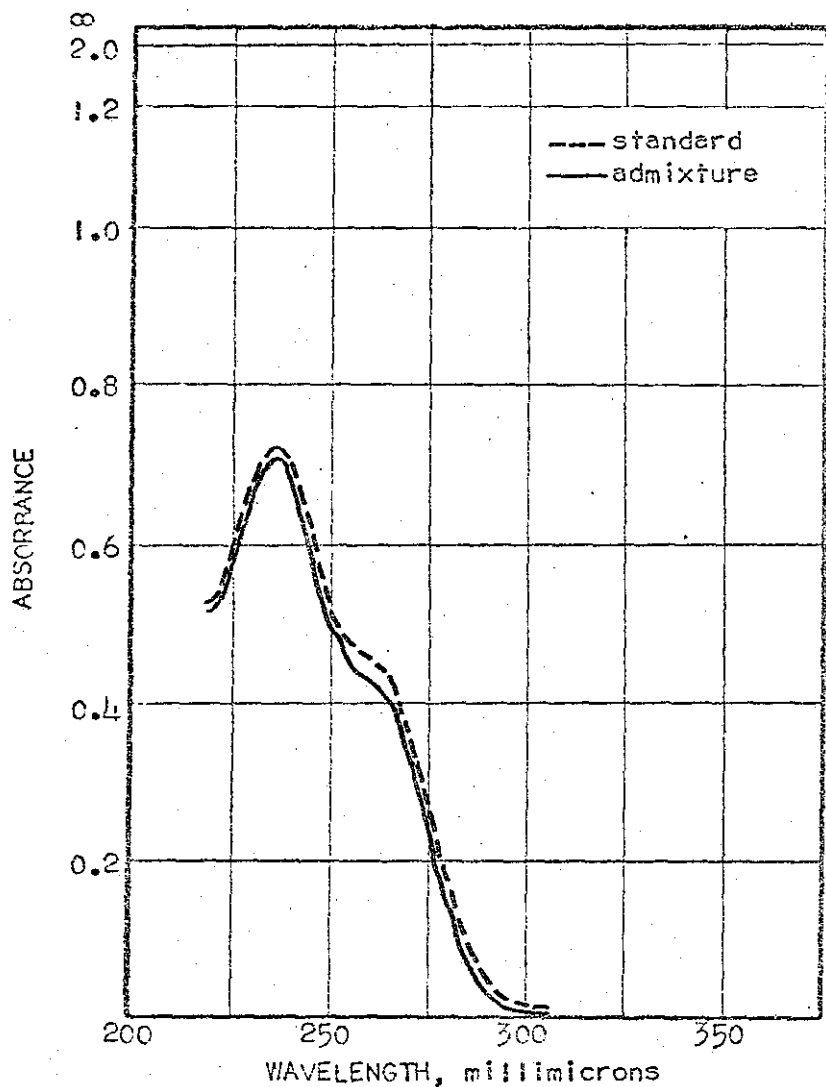


Sodium Cephalothin 20 mcg./ml.
Ref: 5-Fluorouracil 10 mcg./ml.

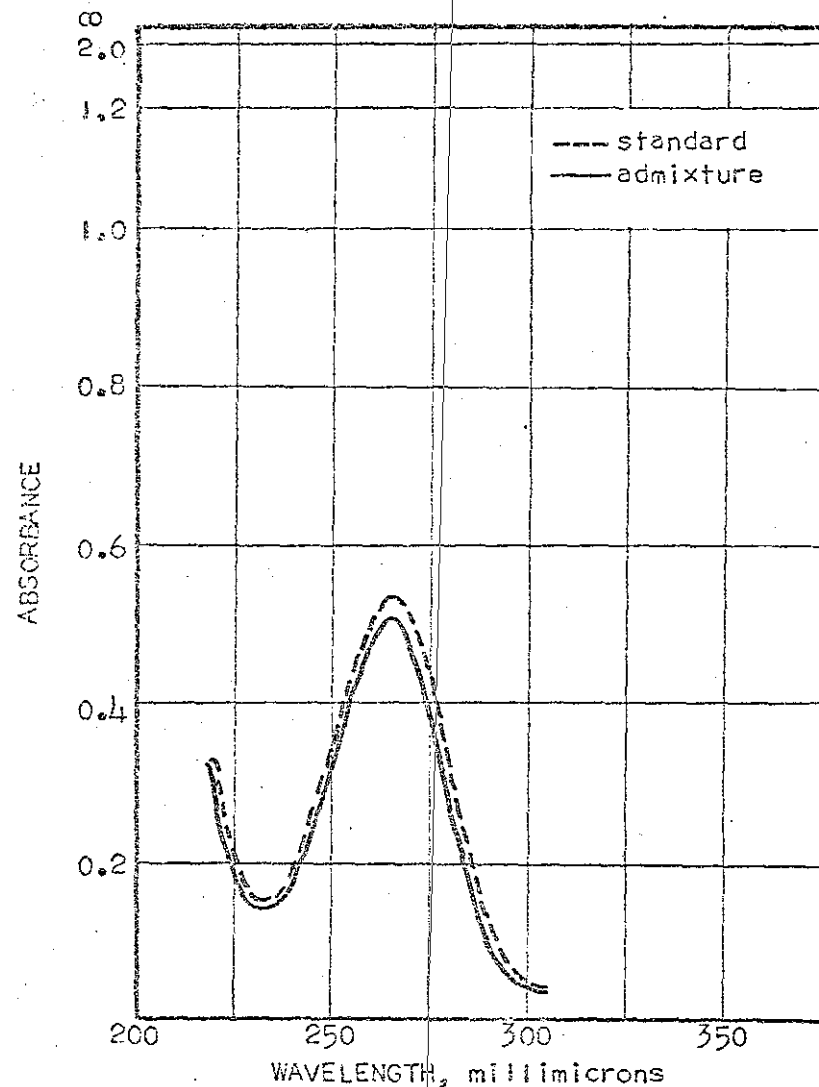


5-Fluorouracil 10 mcg./ml.
Ref: Sodium Cephalothin 20 mcg./ml.

Figure 14. U.V. Spectrum of Sodium Cephalothin (λ_{max} 236 mμ) with 5-Fluorouracil (λ_{max} 265.5 mμ) in 5 percent Dextrose Injection at four hours.



Sodium Cephalothin 20 mcg./ml.
Ref: 5-Fluorouracil 10 mcg./ml.



5-Fluorouracil 10 mcg./ml.
Ref: Sodium Cephalothin 20 mcg./ml.

Figure 15. U.V. Spectrum of Sodium Cephalothin (λ_{max} 236 mμ) with 5-Fluorouracil (λ_{max} 265.5 mμ) in 5 percent Dextrose Injection at eight hours.

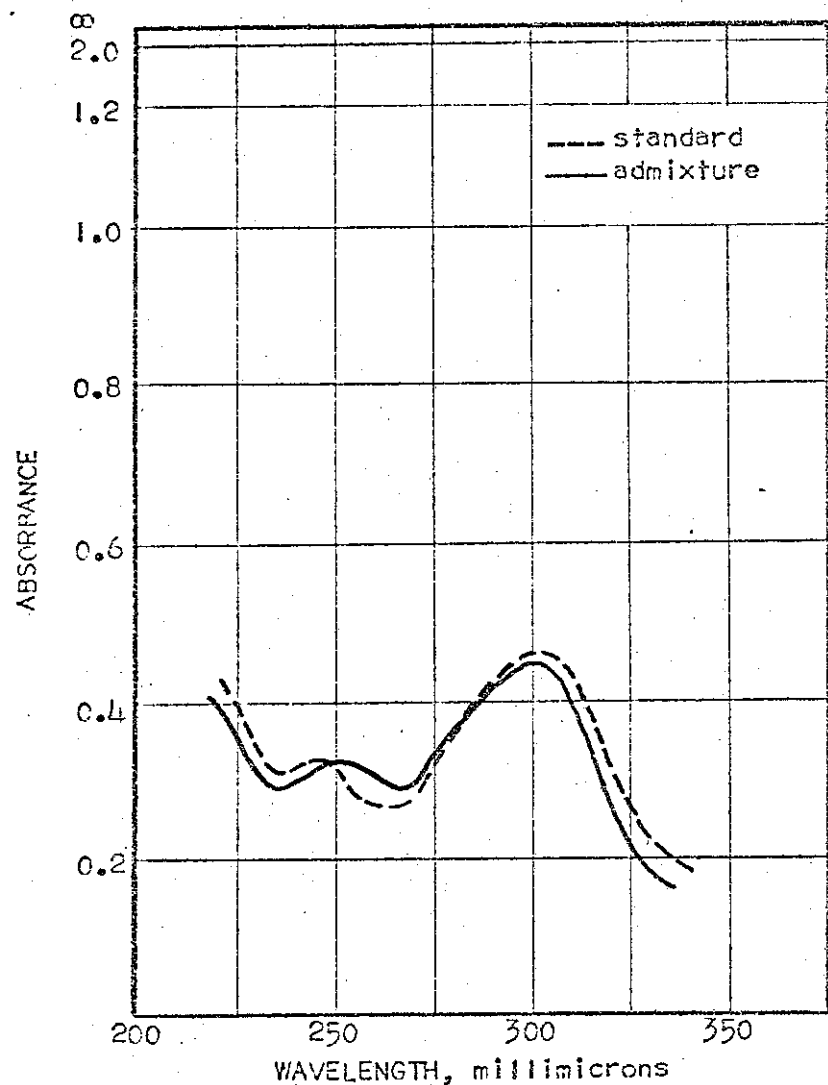
ADMIXTURE #6 PREDNISOLONE SODIUM PHOSPHATE--METHOTREXATE

Preparation of the following admixture consisted of adding prednisolone sodium phosphate, 200 mcg./ml. to methotrexate sodium 200 mcg./ml. As before, both were considered therapeutic concentrations that might be used in the clinical situation.

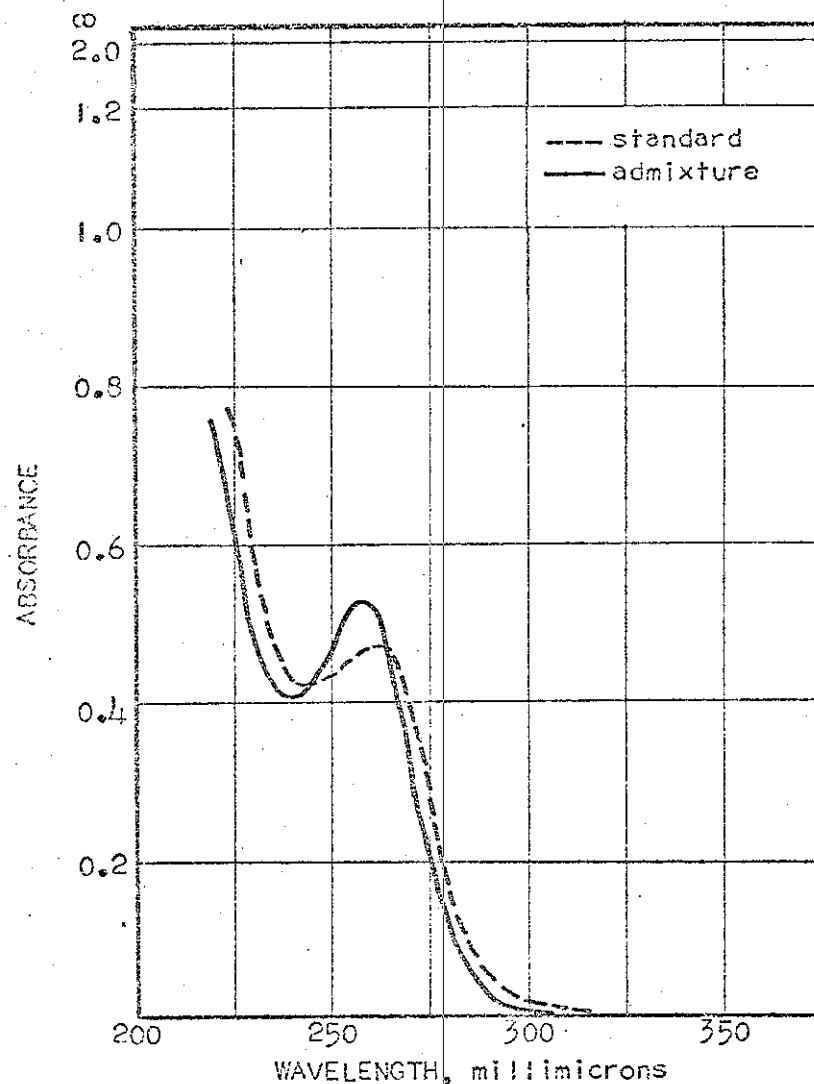
Again, due to the limitations of the spectrophotometer in preventing the use of these solutions directly, appropriate dilutions were made in order to obtain an absorbance spectrum for each of the drugs in the admixture. The dilutions resulted in the following concentrations for each of the drugs: prednisolone sodium phosphate, 8 mcg./ml. and methotrexate sodium, 8 mcg./ml. These dilutions were done from the therapeutic admixtures just prior to each of the readings at the 1, 4, and 8 hour intervals.

Analysis of the admixtures through the use of the spectrophotometer revealed no significant change in the absorbance of methotrexate sodium comparing the primary peak of the standard with the primary peak of the reference spectrum. There was, however, an alteration or shift in a secondary peak of the methotrexate sodium spectrum. For the prednisolone sodium phosphate, the absorbance increased unexpectedly when in the admixture. Both alterations occurred in the initial admixture and remained throughout the eight hour period. (Figures 16, 17, 18) These changes would be indicative of a change in the chemical nature or structural integrity of the additives and should be

considered to be a chemical incompatibility. There were no visual signs of any "physical" incompatibility.

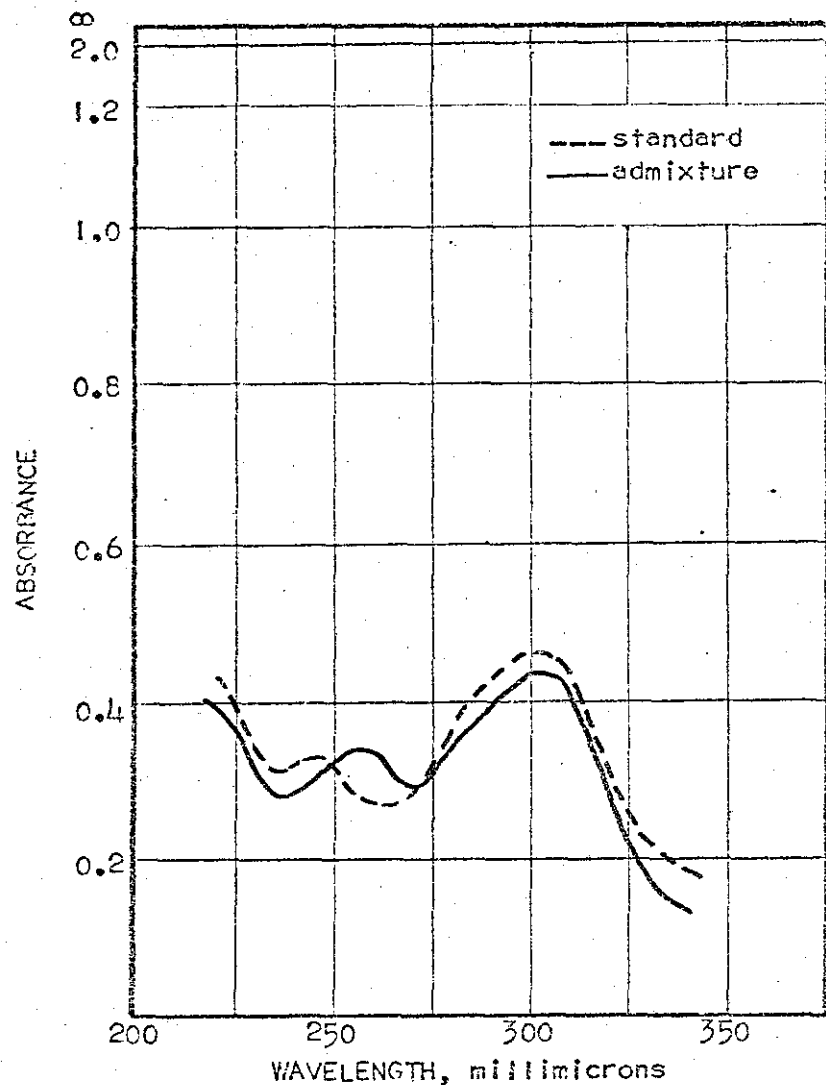


Methotrexate Sodium 8 mcg./ml.
 Ref: Prednisolone Sodium Phosphate
 8 mcg./ml.

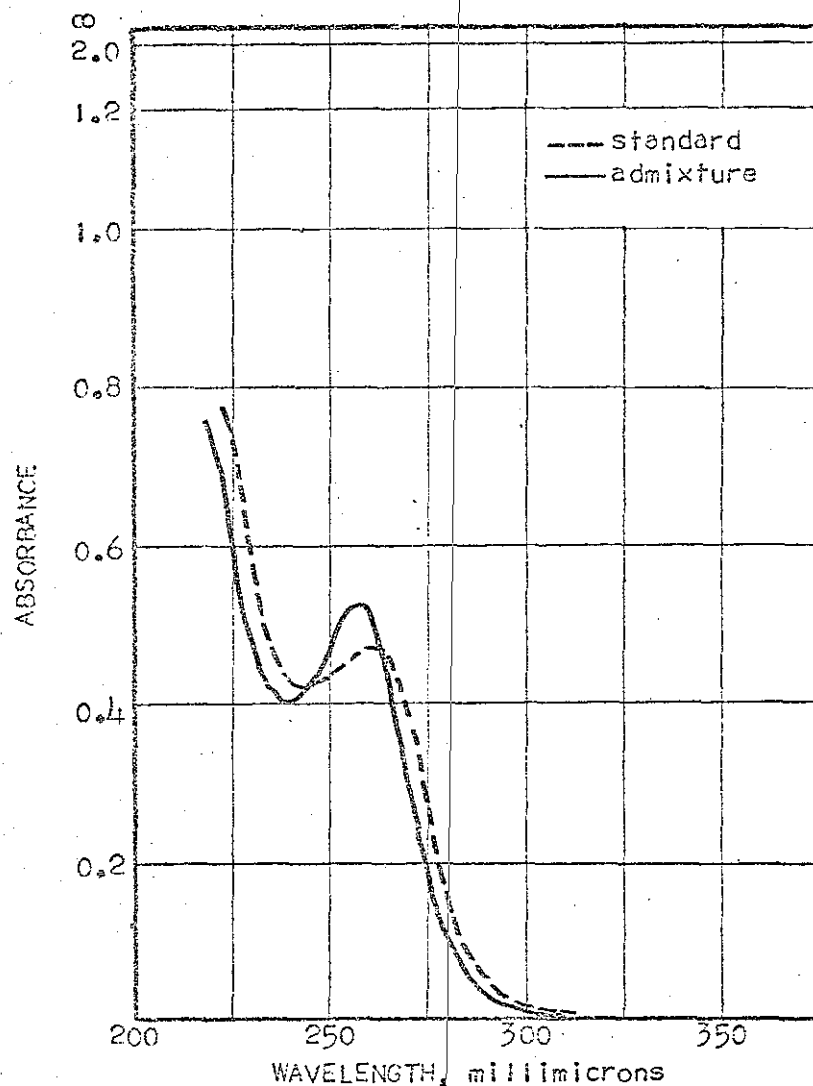


Prednisolone Sodium Phosphate 8 mcg./ml.
 Ref: Methotrexate Sodium 8 mcg./ml.

Figure 16. U.V. Spectrum of Methotrexate Sodium (λ_{\max} 302 mμ) with Prednisolone Sodium Phosphate (λ_{\max} 257 mμ) in 5 percent Dextrose Injection at one hour.

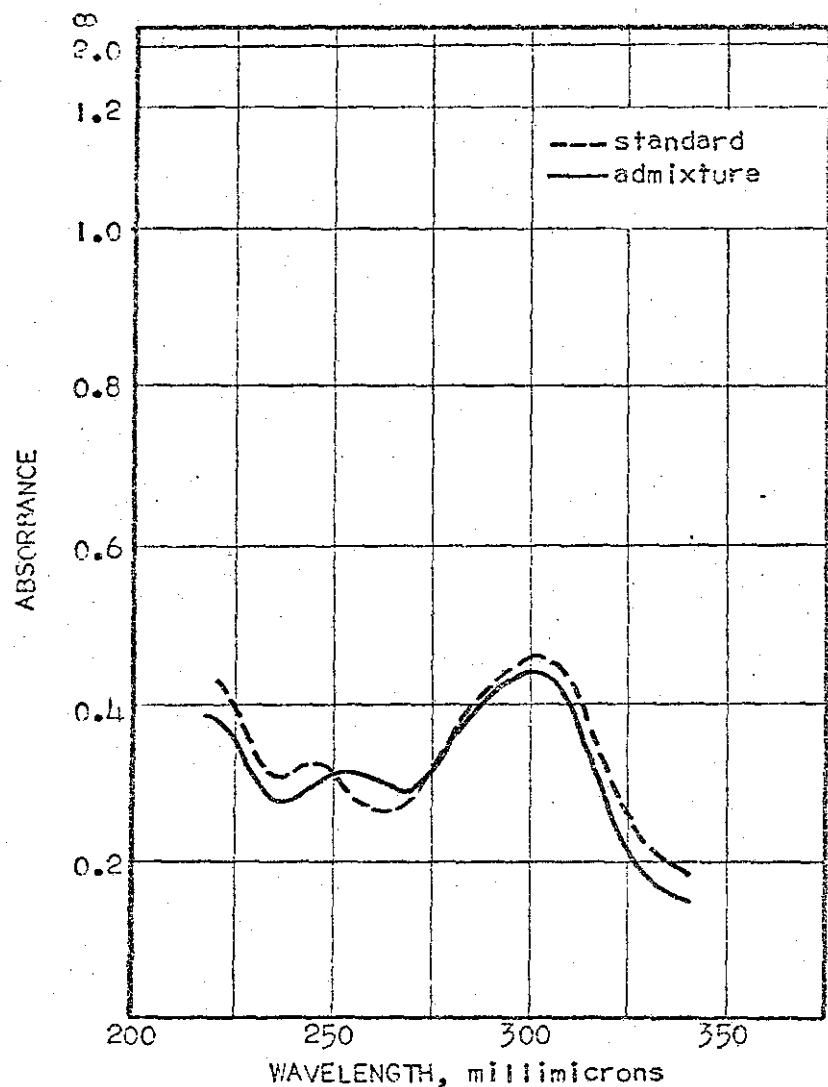


Methotrexate Sodium 8 mcg./ml.
Ref: Prednisolone Sodium Phosphate

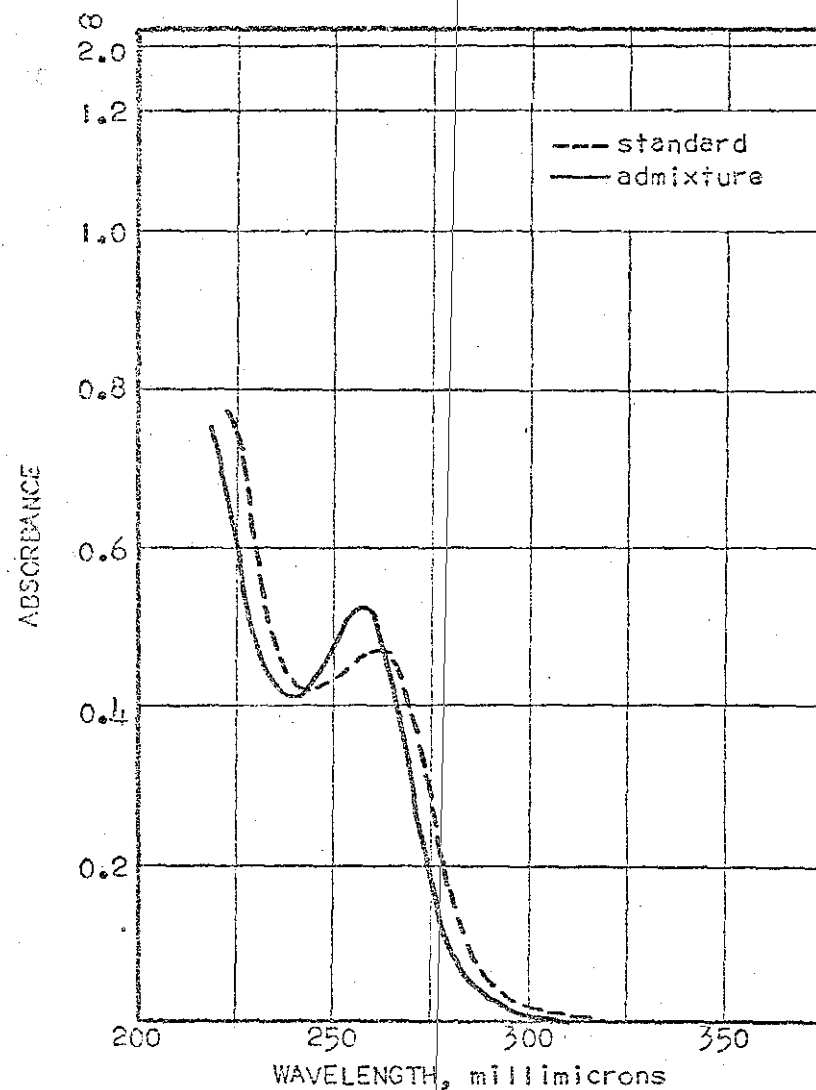


Prednisolone Sodium Phosphate 8 mcg./ml.
Ref: Methotrexate Sodium 8 mcg./ml.

Figure 17. U.V. Spectrum of Methotrexate Sodium (λ_{max} 302 mμ) with Prednisolone Sodium Phosphate (λ_{max} 257 mμ) in 5 percent Dextrose Injection at four hours.



Methotrexate Sodium 8 mcg./ml.
 Ref: Prednisolone Sodium Phosphate
 8 mcg./ml.



Prednisolone Sodium Phosphate 8 mcg./ml.
 Ref: Methotrexate Sodium 8 mcg./ml.

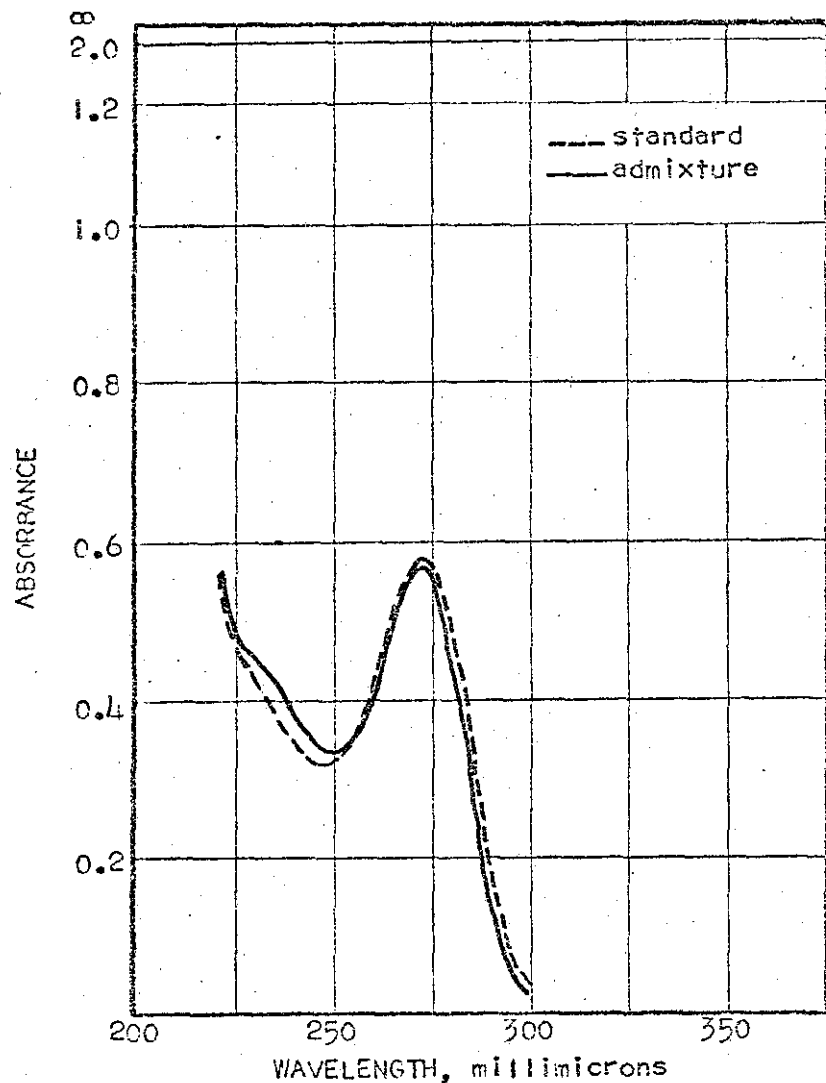
Figure 18. U.S. Spectrum of Methotrexate Sodium (λ_{max} 302 mμ) with Prednisolone Sodium Phosphate (λ_{max} 257 mμ) in 5 percent Dextrose Injection at eight hours.

ADMIXTURE #7 METHOTREXATE SODIUM--CYTARABINE

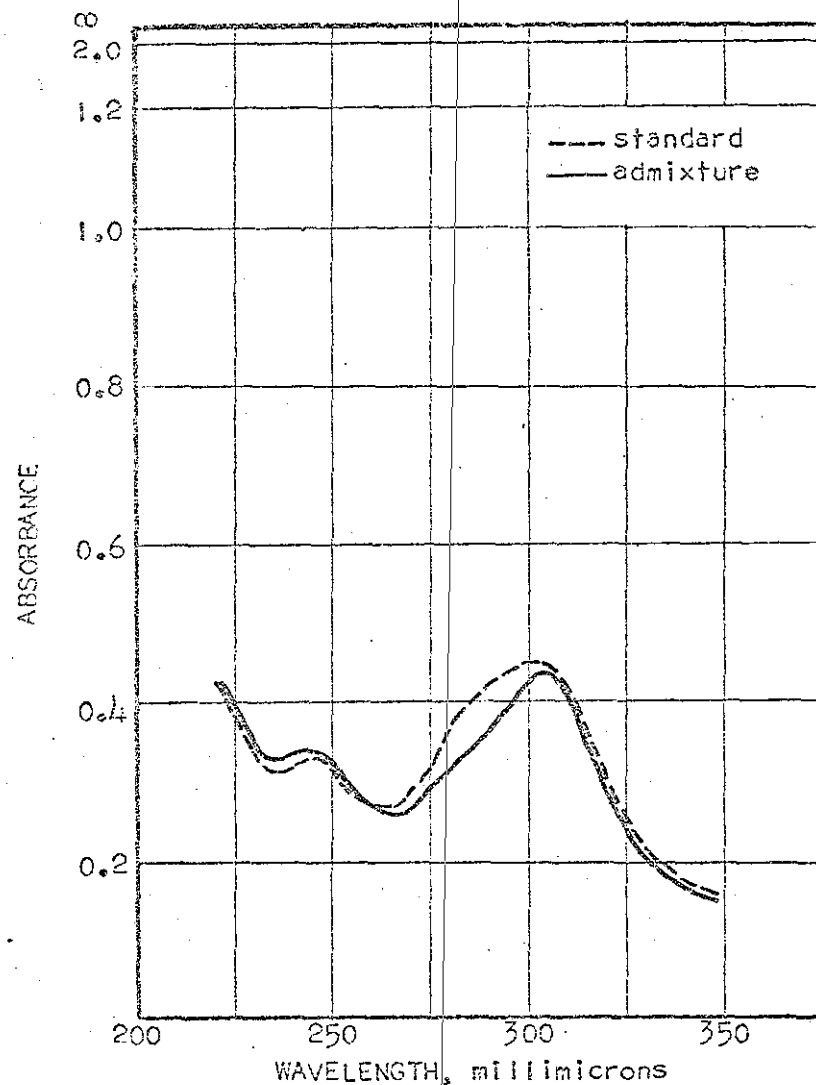
Again using Dextrose 5 percent Injection as the vehicle, methotrexate sodium was added to cytarabine using therapeutic concentrations of 200 mcg./ml. and 400 mcg./ml. respectively.

Appropriate dilutions were made so that the absorbance of each drug would be within the range that could be scanned by the spectrophotometer. The concentrations of the dilutions were the following: methotrexate sodium, 8 mcg./ml. and cytarabine 16 mcg./ml. These dilutions were made from the therapeutic admixtures just prior to the 1, 4, and 8 hour recordings.

While slight alterations in the spectrum of methotrexate sodium were observed, the changes did not appear to be significant. There did appear to be a slight shift in the λ_{max} of the drug; however, this change was somewhat questionable. There was no apparent change in the spectrum or in the absorbance of cytarabine. (Figures 19, 20, 21)

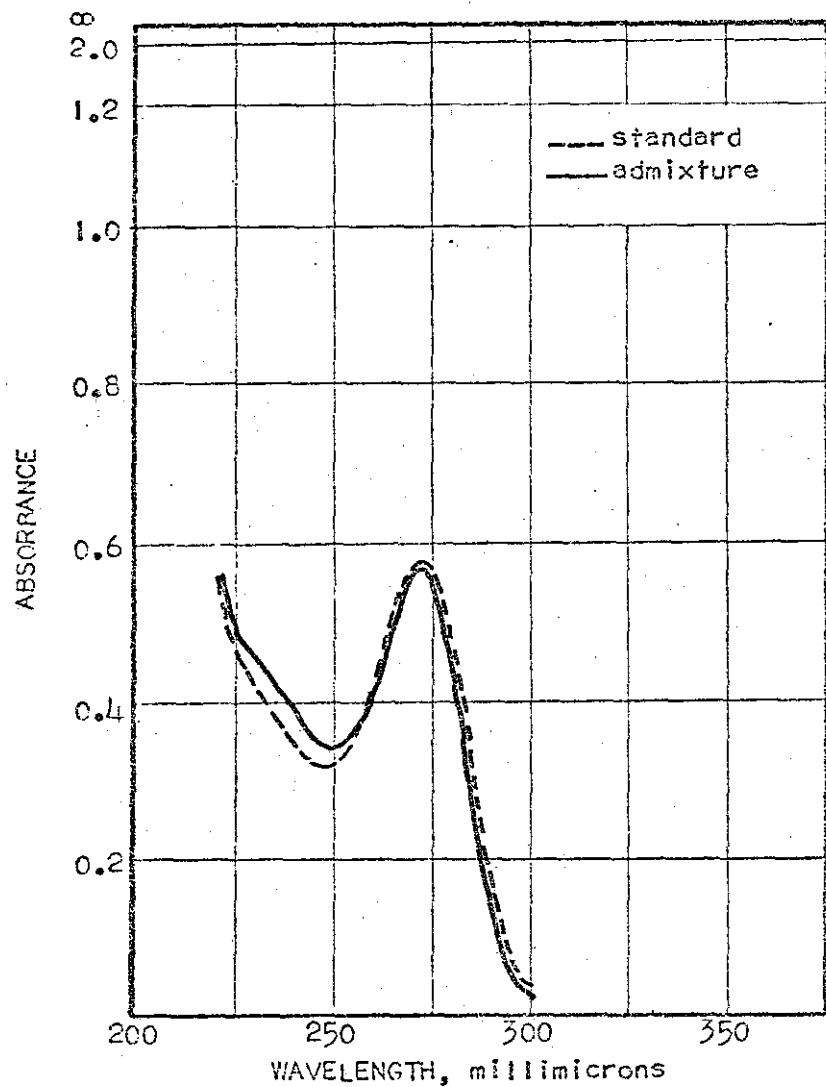


Cytarabine 16 mcg./ml.
Ref: Methotrexate Sodium 8 mcg./ml.

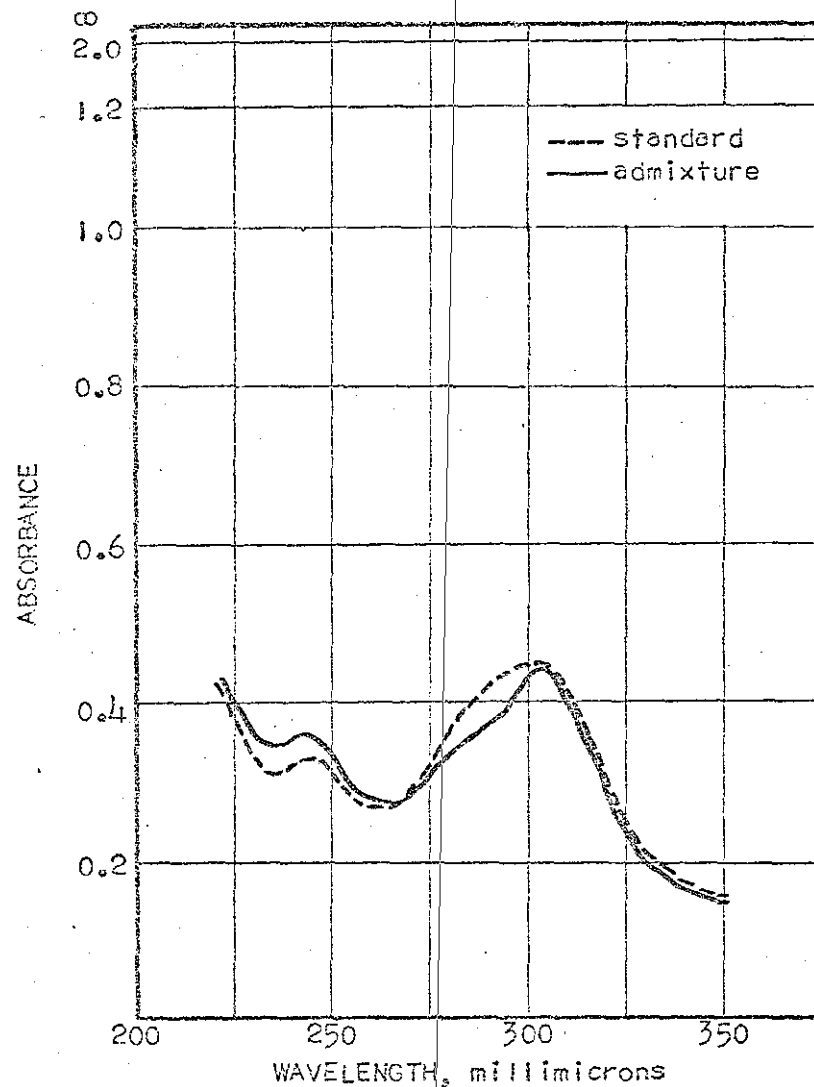


Methotrexate Sodium 8 mcg./ml.
Ref: Cytarabine 16 mcg./ml.

Figure 19. U.V. Spectrum of Cytarabine (λ_{\max} 272 mμ) with Methotrexate Sodium (λ_{\max} 302 mμ) in 5 percent Dextrose Injection at one hour.

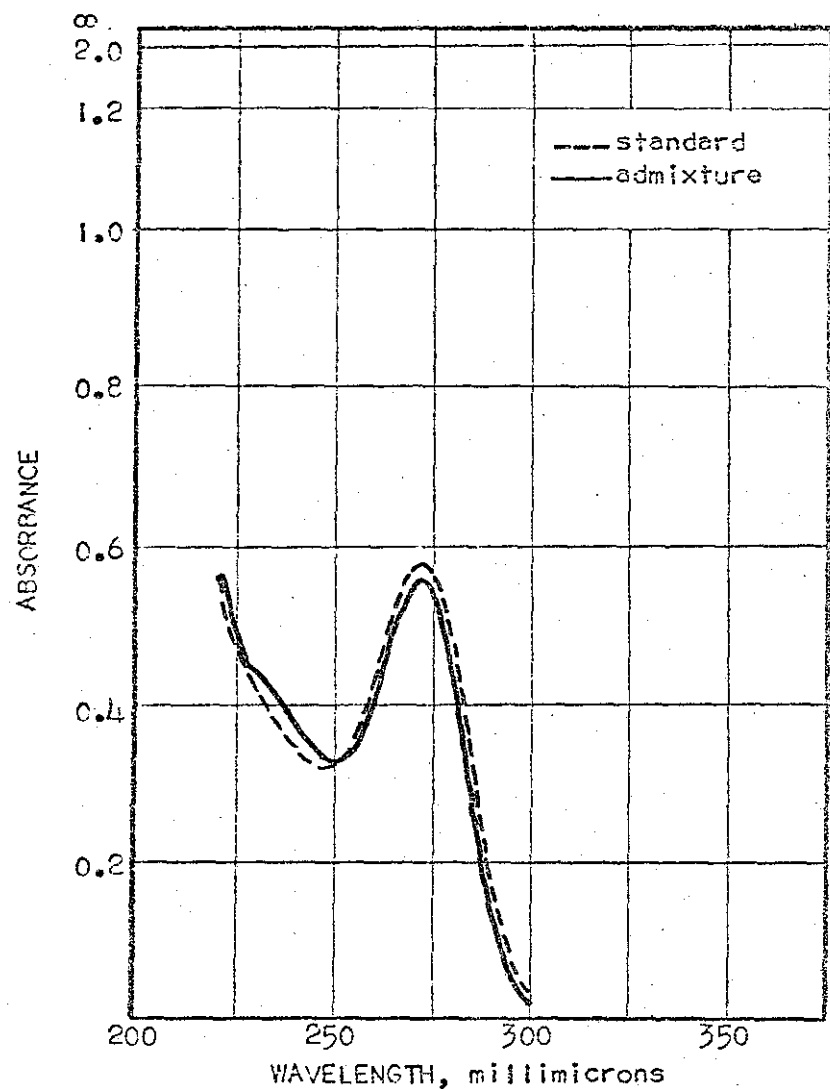


Cytarabine 16 mcg./ml.
Ref: Methotrexate Sodium 8 mcg./ml.

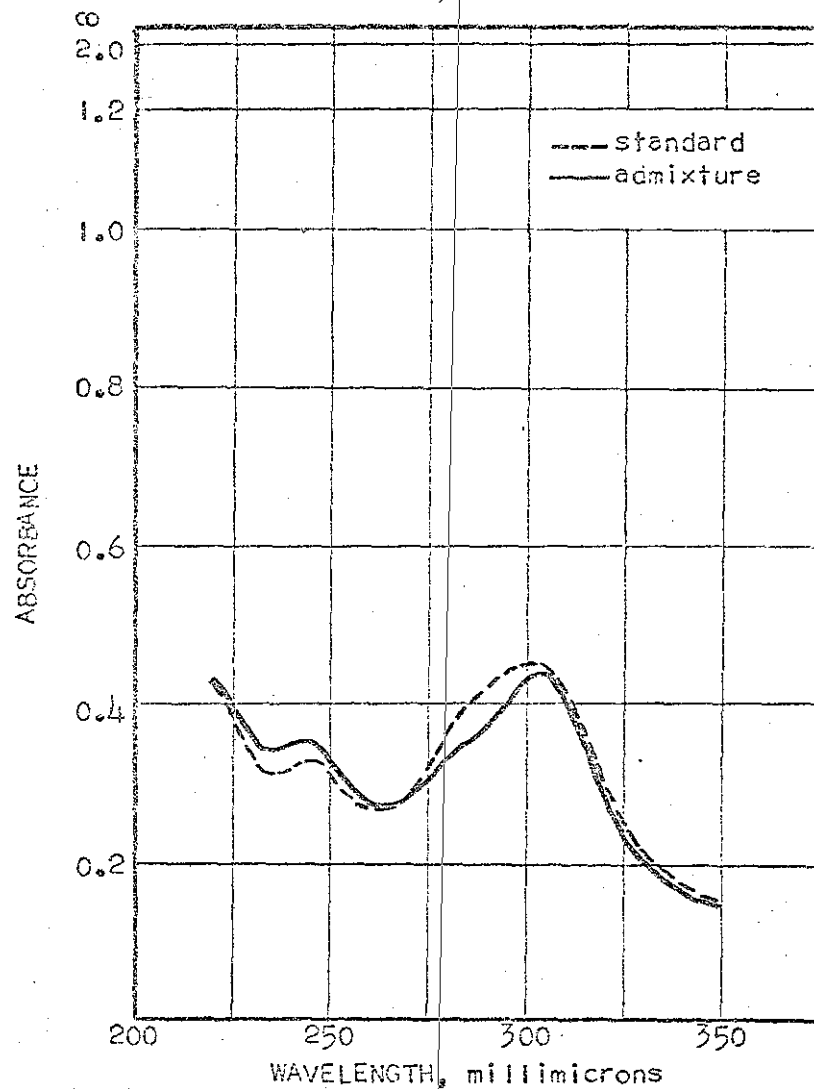


Methotrexate Sodium 8 mcg./ml.
Ref: Cytarabine 16 mcg./ml.

Figure 20. U.V. Spectrum of Cytarabine (λ_{\max} 272 mμ) with Methotrexate Sodium (λ_{\max} 302 mμ) in 5 percent Dextrose Injection at four hours.



Cytarabine 16 mcg./ml.
Ref: Methotrexate Sodium 8 mcg./ml.



Methotrexate Sodium 8 mcg./ml.
Ref: Cytarabine 16 mcg./ml.

Figure 21. U.V. Spectrum of Cytarabine (λ_{\max} 272 mμ) with Methotrexate Sodium (λ_{\max} 302 mμ) in 5 percent Dextrose Injection at eight hours.

ADMIXTURE #8 CYTARABINE---SODIUM CEPHALOTHIN

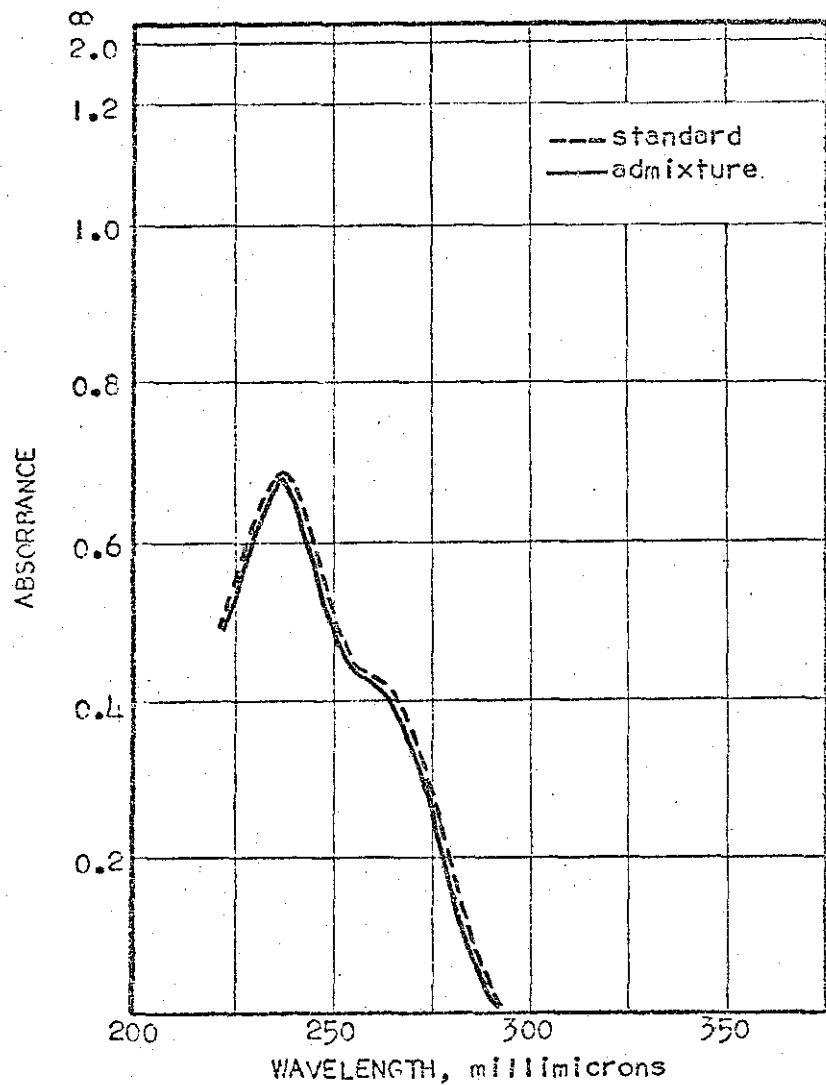
In the following admixture, cytarabine was added to sodium cephalothin. The therapeutic concentrations used were the following: cytarabine 800 mcg./ml. and sodium cephalothin 1,000 mcg./ml. The concentration in this admixture for cytarabine was double that which had been used in previous admixtures. This was done because ~~of the large concentration used for the sodium cephalothin~~ which was necessary to simulate normal therapeutic concentrations of that drug. To facilitate dilutions that were necessary, it was required to use a concentration of cytarabine that at least somewhat approached that of the sodium cephalothin.

After preparing the dilutions to obtain an ultra-violet spectrum for each of the two drugs in the admixture, the resulting concentrations were 16 mcg./ml. for the cytarabine and 20 mcg./ml. for the sodium cephalothin.

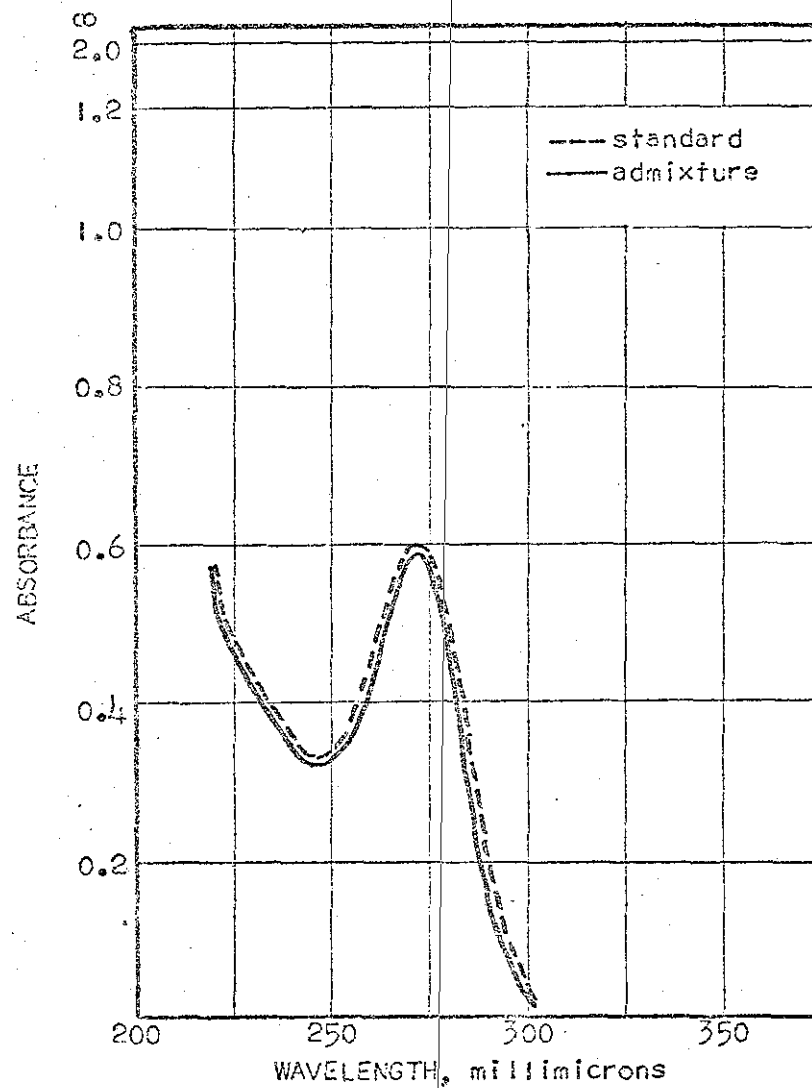
Comparison of the absorption spectrum of each of the additives in the admixture with the respective reference spectra demonstrated no alteration in shape nor any appreciable loss of absorbance. Throughout the eight hour period there was a slight decrease in the absorbance for cytarabine; however, this was not significant when evaluated in terms of the Beer plot.

The data obtained from this admixture would seem to indicate the absence of any chemical interaction between these two drugs and this admixture would appear to be

compatible. (Figures 22, 23, 24)

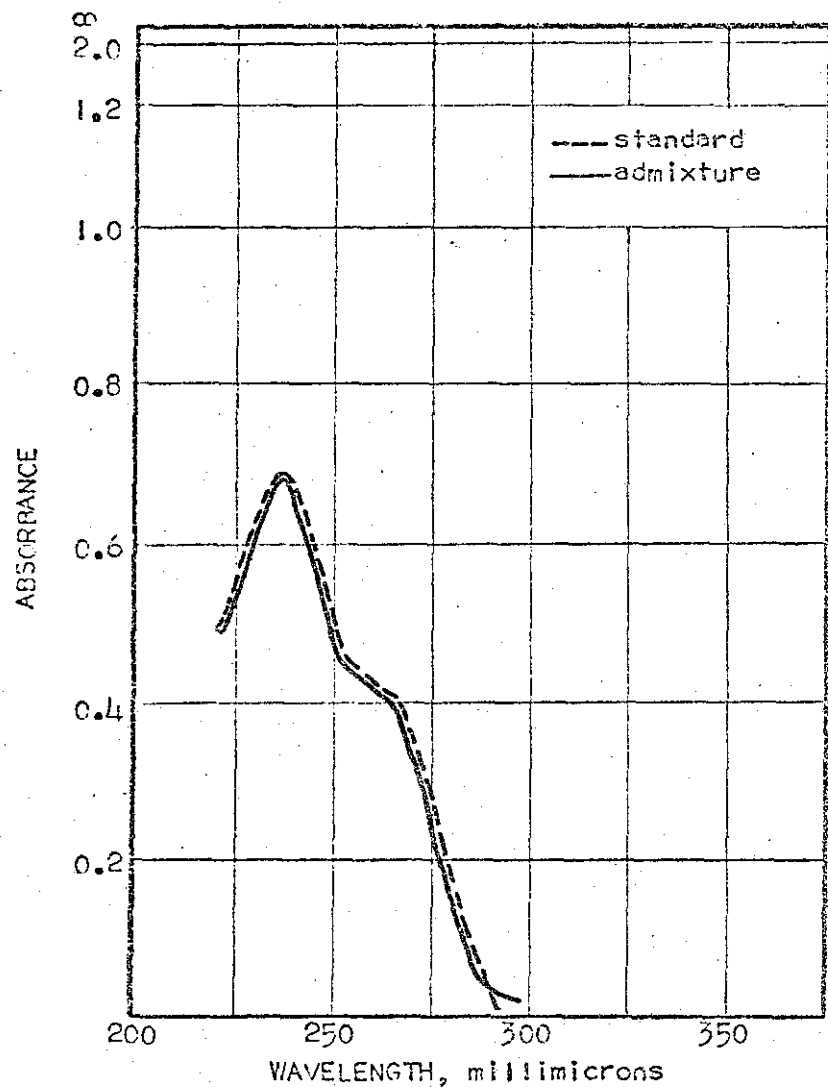


Sodium Cephalothin 20 mcg./ml.
Ref: Cytarabine 16 mcg./ml.

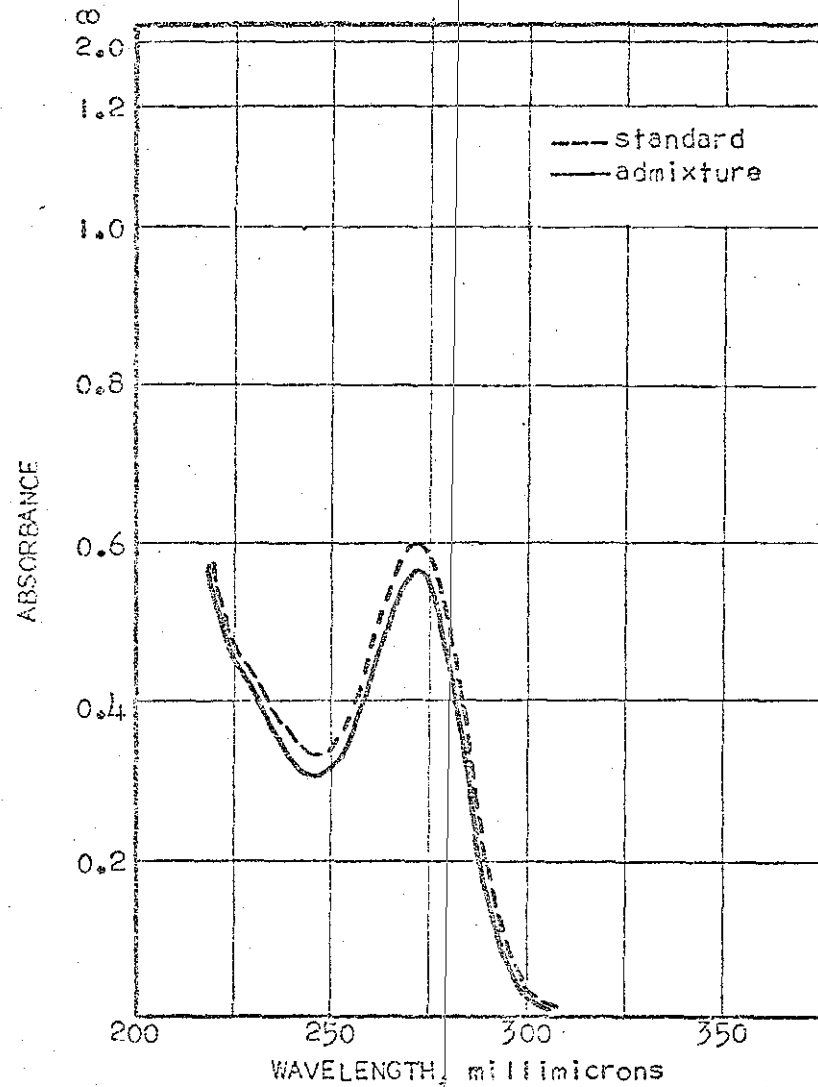


Cytarabine 16 mcg./ml.
Ref: Sodium Cephalothin 20 mcg./ml.

Figure 22. U. V. Spectrum of Sodium Cephalothin (λ_{max} 236 mμ) with Cytarabine (λ_{max} 272 mμ) in 5 percent Dextrose Injection at one hour.

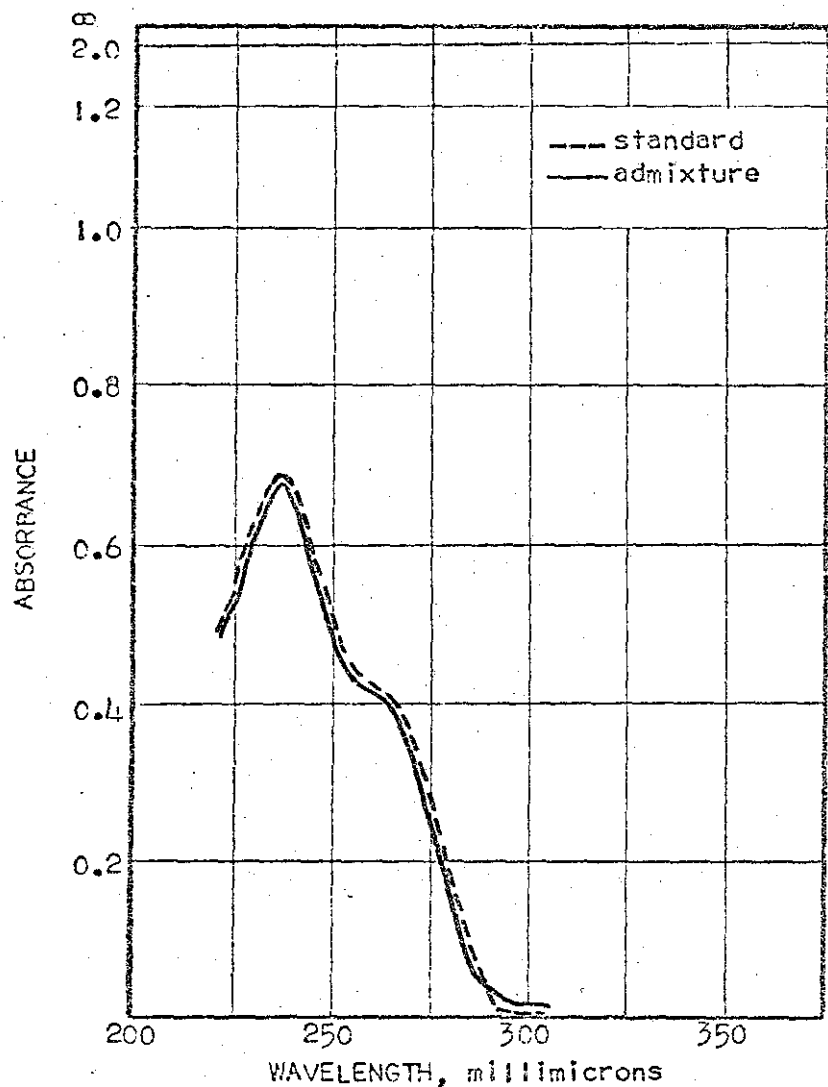


Sodium Cephalothin 20 mcg./ml.
Ref: Cytarabine 16 mcg./ml.

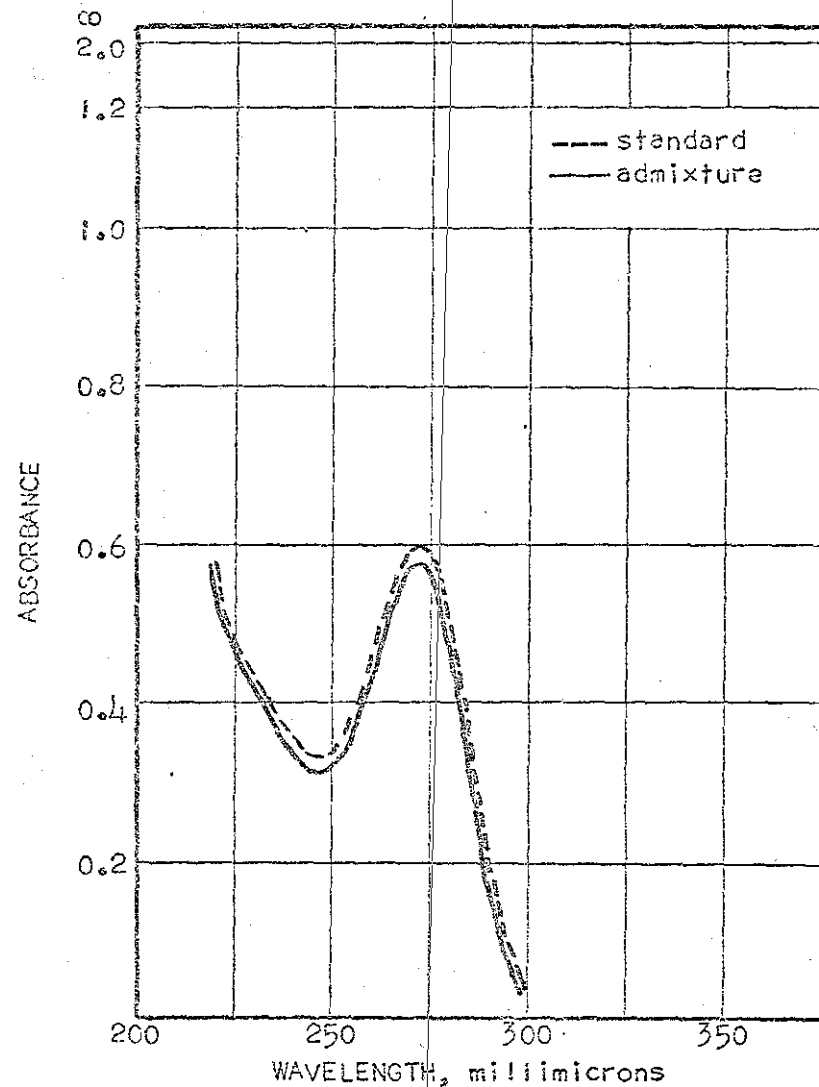


Cytarabine 16 mcg./ml.
Ref: Sodium Cephalothin 20 mcg./ml.

Figure 23. U.V. Spectrum of Sodium Cephalothin (λ max 236 mμ) with Cytarabine (λ max 272 mμ) in 5 percent Dextrose Injection at four hours.



Sodium Cephalothin 20 mcg./ml.



Cytarabine 16 mcg./ml.
Ref: Sodium Cephalothin 20 mcg./ml.

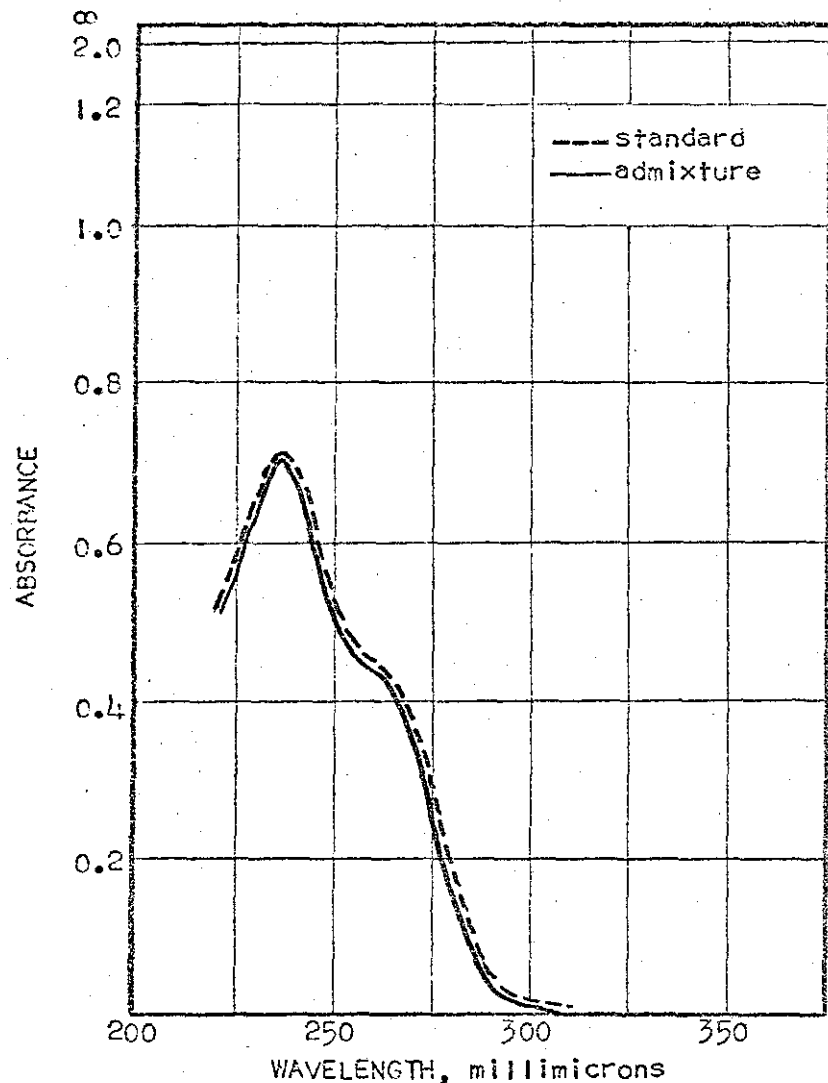
Figure 24. U.V. Spectrum of Sodium Cephalothin (λ_{\max} 236 mμ) with Cytarabine (λ_{\max} 272 mμ) in 5 percent Dextrose Injection at eight hours.

ADMIXTURE #9 PREDNISOLONE SODIUM PHOSPHATE---SODIUM CEPHALOTHIN

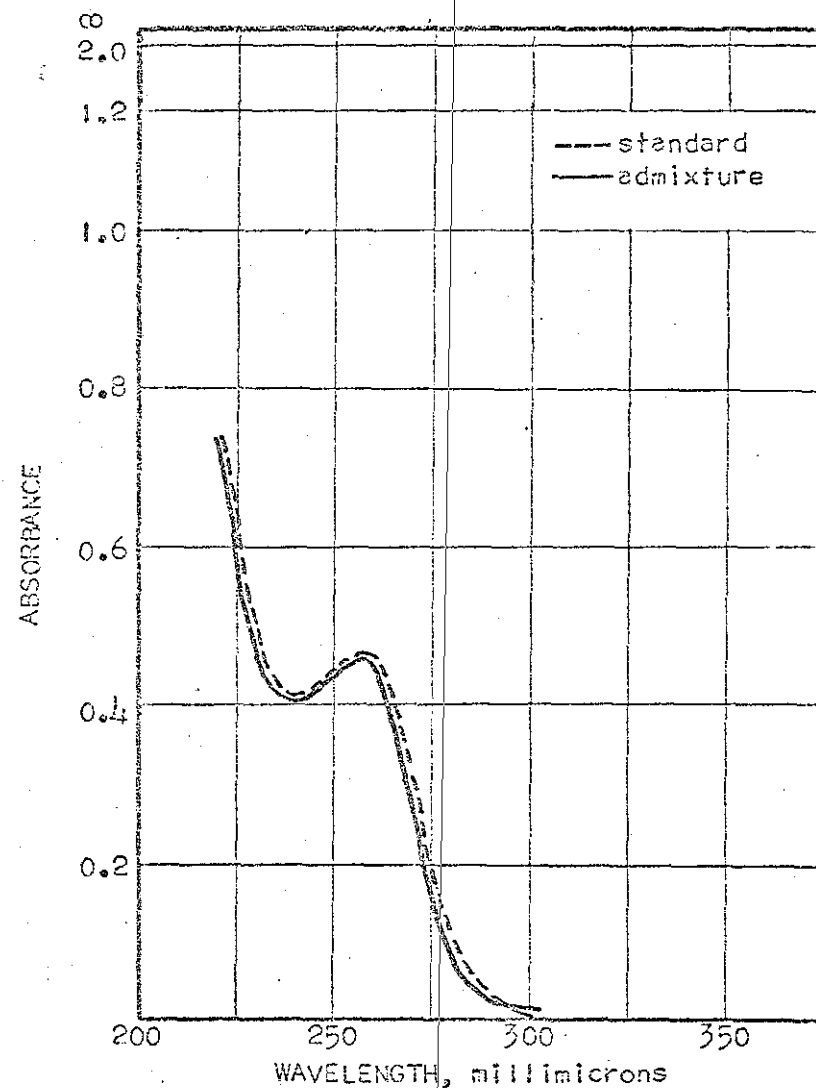
An admixture containing therapeutic concentrations of prednisolone sodium phosphate, 400 mcg./ml. and sodium cephalothin, 1,000 mcg./ml. was prepared by adding the prednisolone sodium phosphate to the sodium cephalothin in 5 percent Dextrose Injection. As mentioned in the description of admixture #5, it was necessary to increase the concentration of the prednisolone sodium phosphate to facilitate dilution of the sodium cephalothin to a concentration that could be scanned. The concentration of the prednisolone sodium phosphate used was double that was used in the earlier admixtures.

To obtain the spectrum for each of the drugs, appropriate dilutions were made to achieve a final concentration of 8 mcg./ml. for the prednisolone sodium phosphate and 20 mcg./ml. for the sodium cephalothin.

An interpretation of the spectra over the eight hour period revealed no alteration in their shapes nor in their absorbances when compared to their respective standards. As there were no noticeable changes, the admixture was assumed to be chemically and physically compatible. (Figures 25, 26, 27)

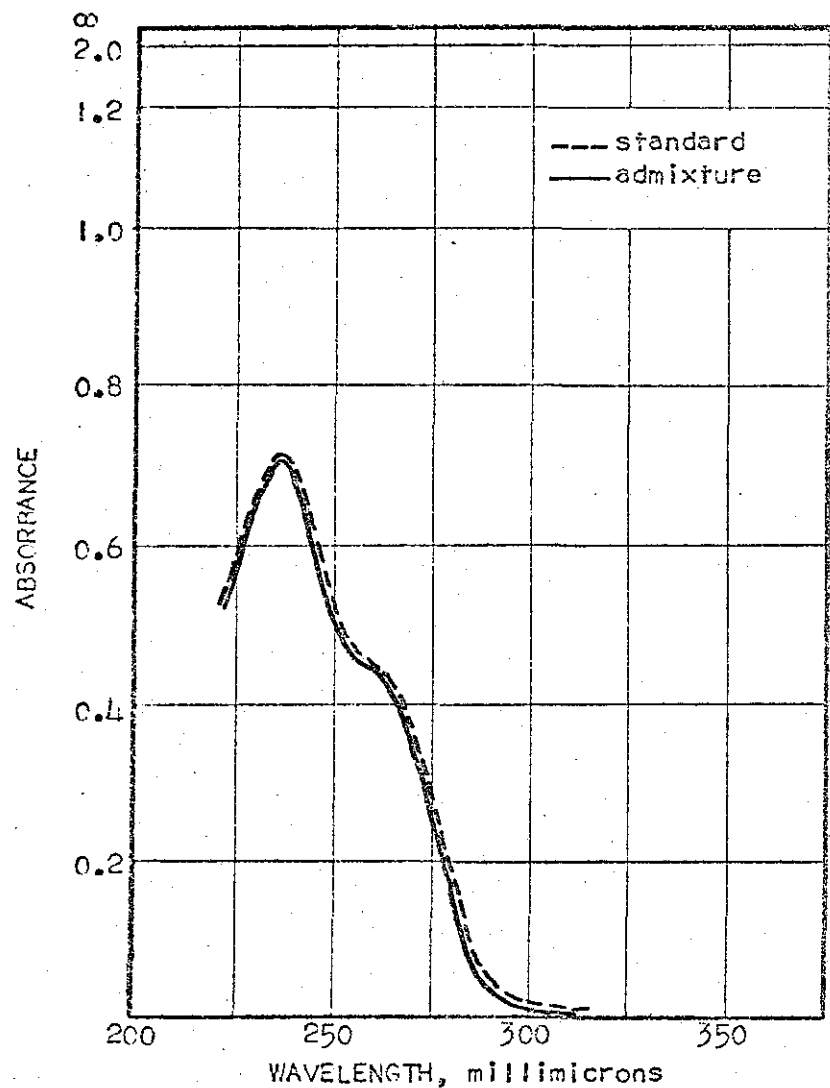


Sodium Cephalothin 20 mcg./ml.
 Ref: Prednisolone Sodium Phosphate
 8 mcg./ml.

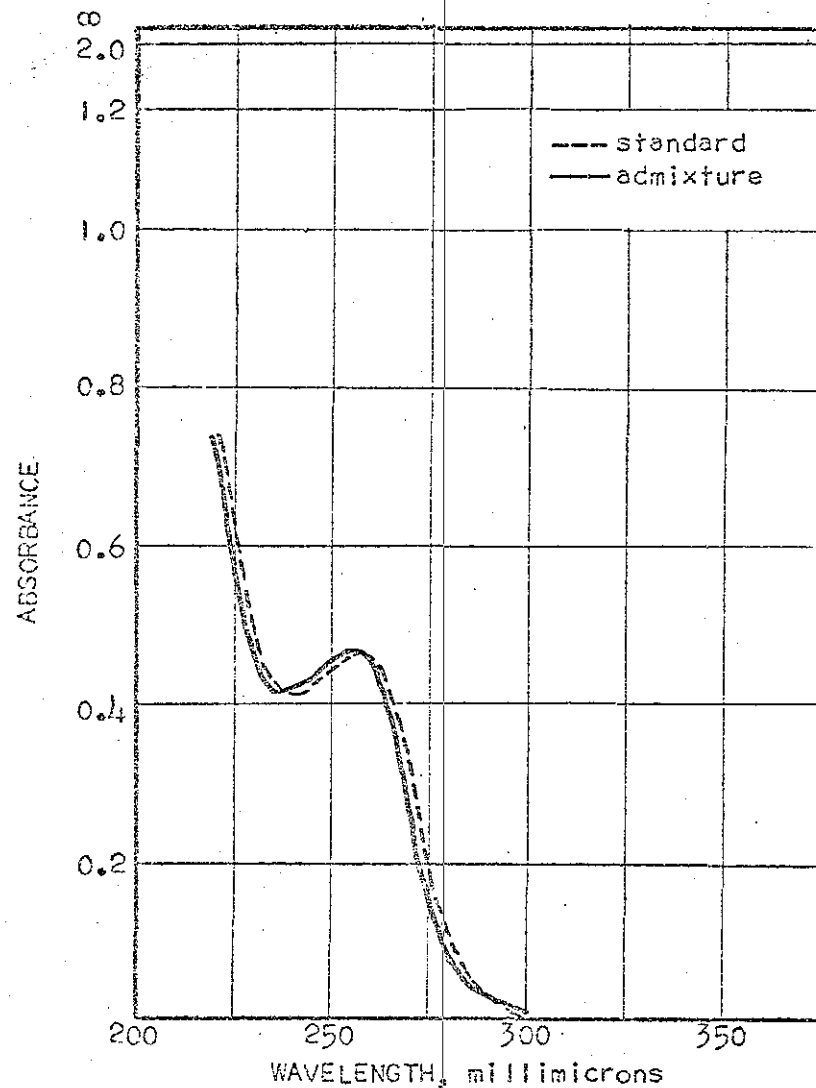


Prednisolone Sodium Phosphate 8 mcg./ml.
 Ref: Sodium Cephalothin 20 mcg./ml.

Figure 25. U.V. Spectrum of Sodium Cephalothin (λ_{\max} 236 mμ) with Cytarabine (λ_{\max} 272 mμ) in 5 percent Dextrose Injection at one hour.

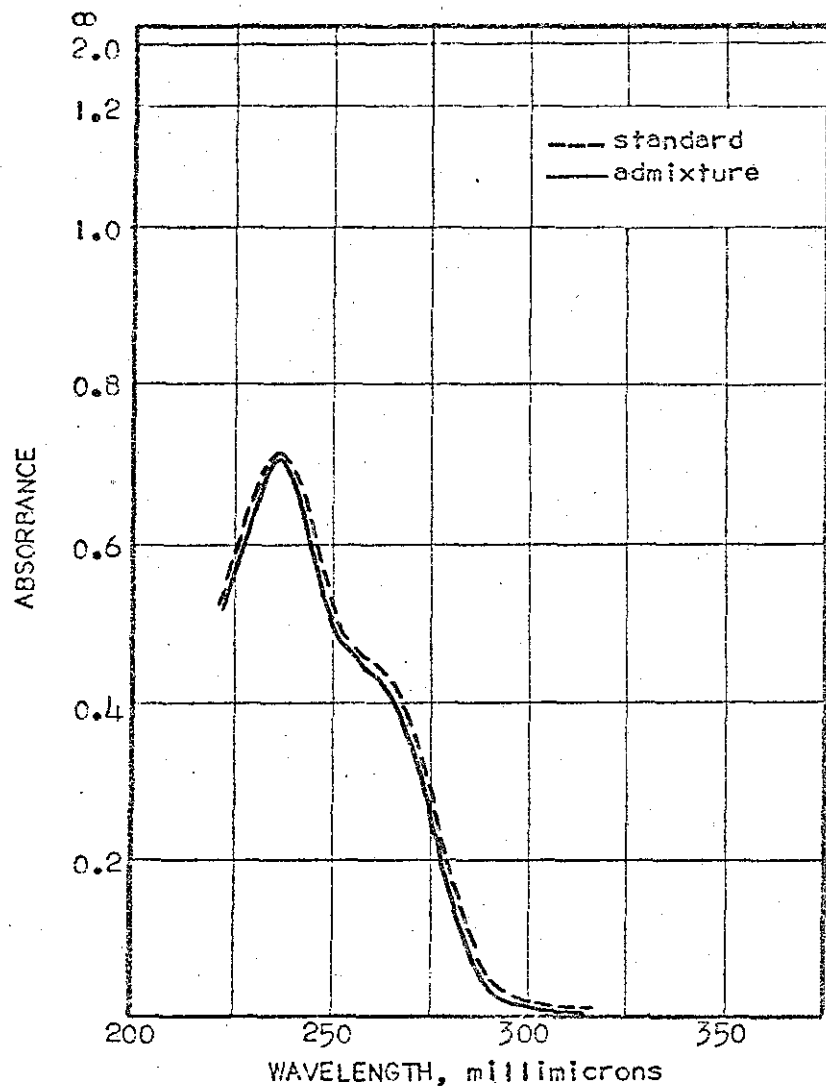


Sodium Cephalothin 20 mcg./ml.
 Ref: Prednisolone Sodium Phosphate
 8 mcg./ml.

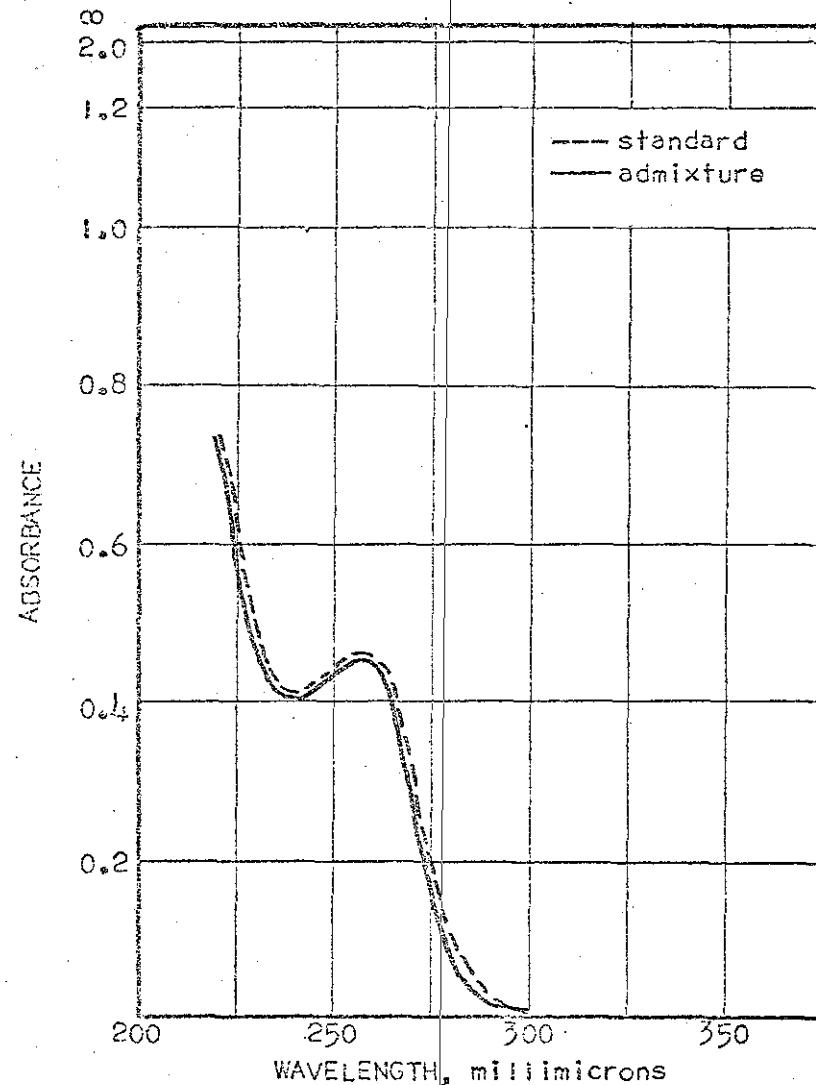


Prednisolone Sodium Phosphate 8 mcg./ml.
 Ref: Sodium Cephalothin 20 mcg./ml.

Figure 26. U.V. Spectrum of Sodium Cephalothin (λ_{\max} 236 m μ) with Cytarabine (λ_{\max} 272 m μ) in 5 percent Dextrose Injection at four hours.



Sodium Cephalothin 20 mcg./ml.
 Ref: Prednisolone Sodium Phosphate
 8 mcg./ml.



Prednisolone Sodium Phosphate 8 mcg./ml.
 Ref: Sodium Cephalothin 20 mcg./ml.

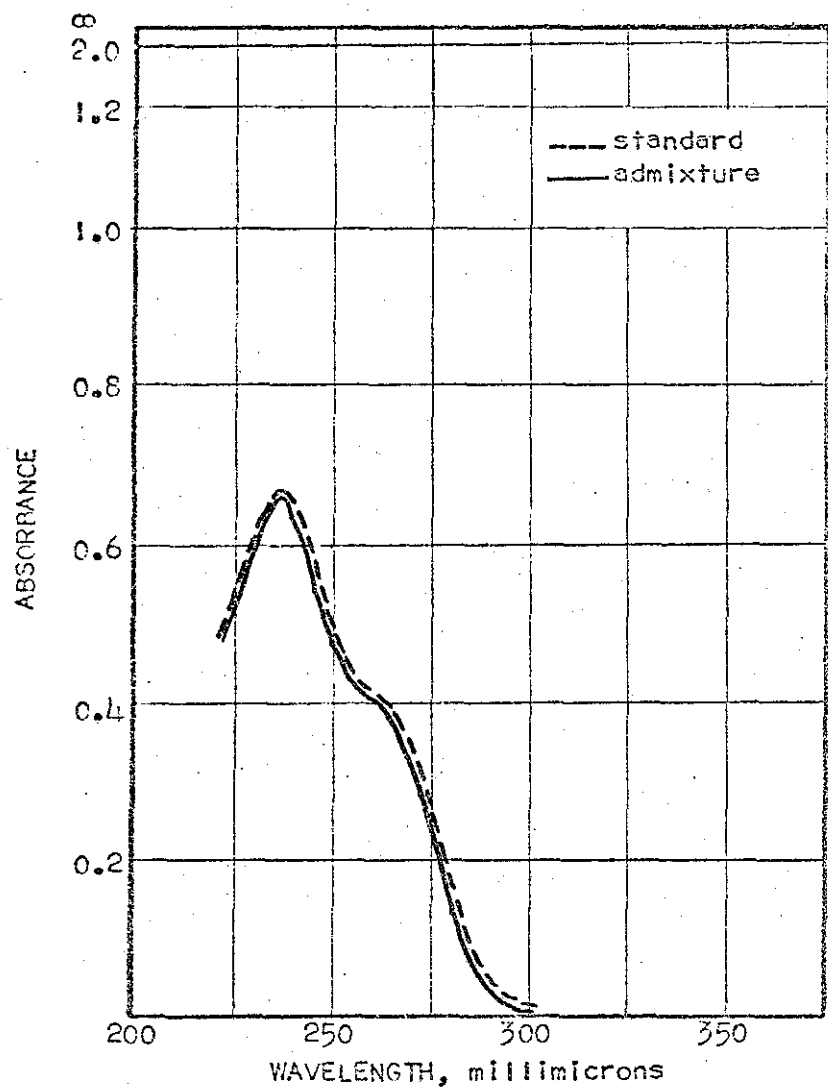
Figure 27. U.V. Spectrum of Sodium Cephalothin (λ_{max} 236 m μ) with Cytarabine (λ_{max} 272 m μ) in 5 percent Dextrose Injection at eight hours.

ADMIXTURE #10 METHOTREXATE SODIUM--SODIUM CEPHALOTHIN

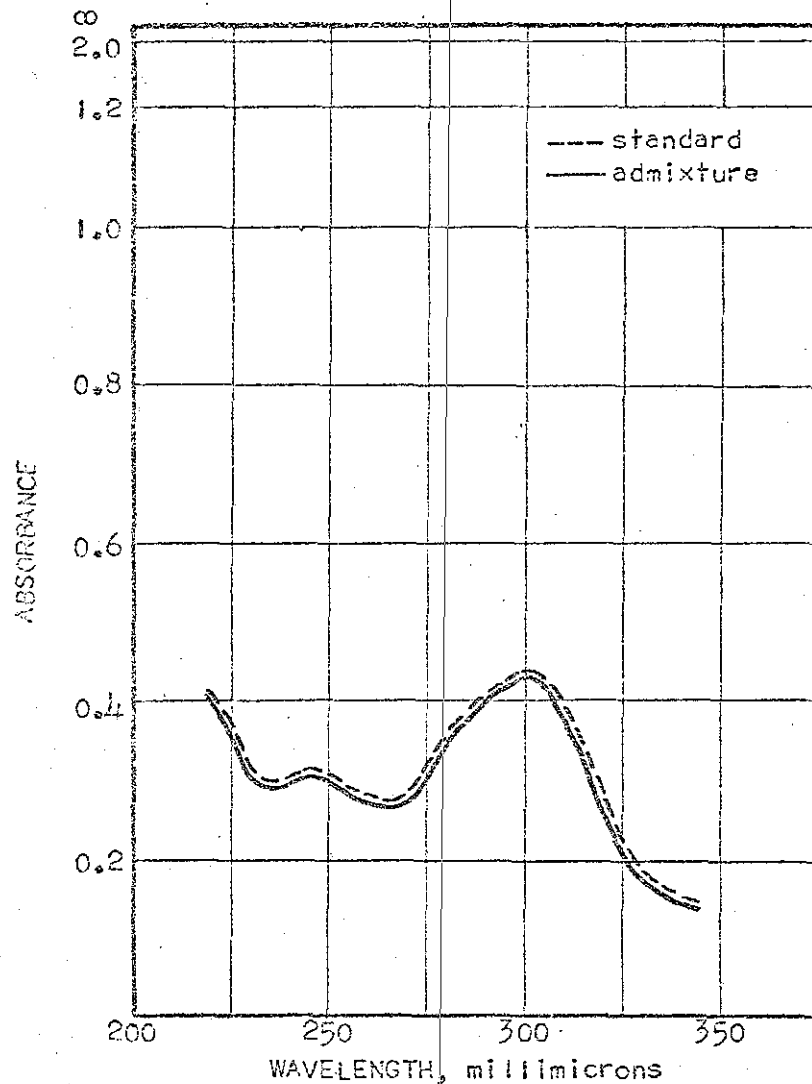
Preparation of the following admixture consisted of adding methotrexate sodium to sodium cephalothin. The concentrations used, which simulated those used in actual clinical medicine, were methotrexate sodium 400 mcg./ml. and sodium cephalothin 1,000 mcg./ml. Again, the concentration for the methotrexate sodium was increased (double that used previously) to approach the unusually high therapeutic concentrations used in medicine with sodium cephalothin.

As was the case in the previous experiments, due to the limitations of the spectrophotometer's preventing the use of the above solutions directly, dilution of the initial admixtures was necessary. The resulting concentrations after final dilution were 8 mcg./ml. for the methotrexate sodium and 20 mcg./ml. for the sodium cephalothin.

During the eight hours of the study, neither spectrum was altered and there was no noticeable decrease in the absorbance, when comparing the spectra with their respective references. (Figures 28, 29, 30)

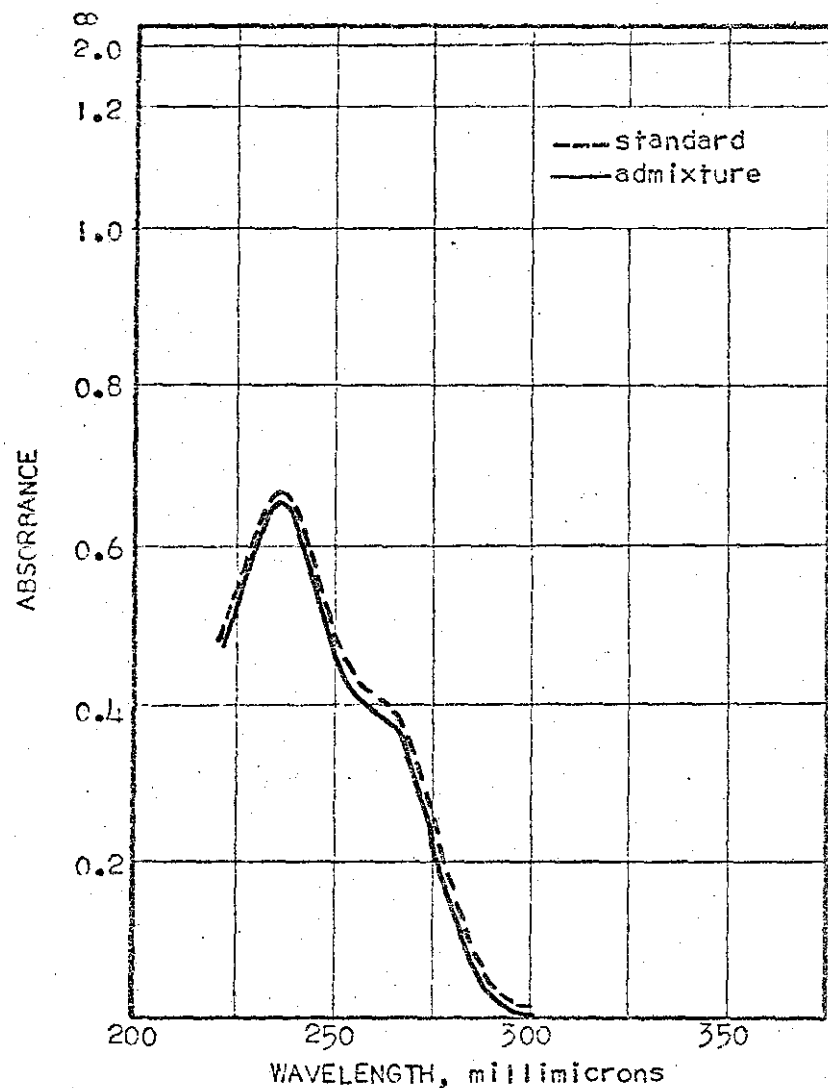


Sodium Cephalothin 20 mcg./ml.
Ref: Methotrexate Sodium 8 mcg./ml.

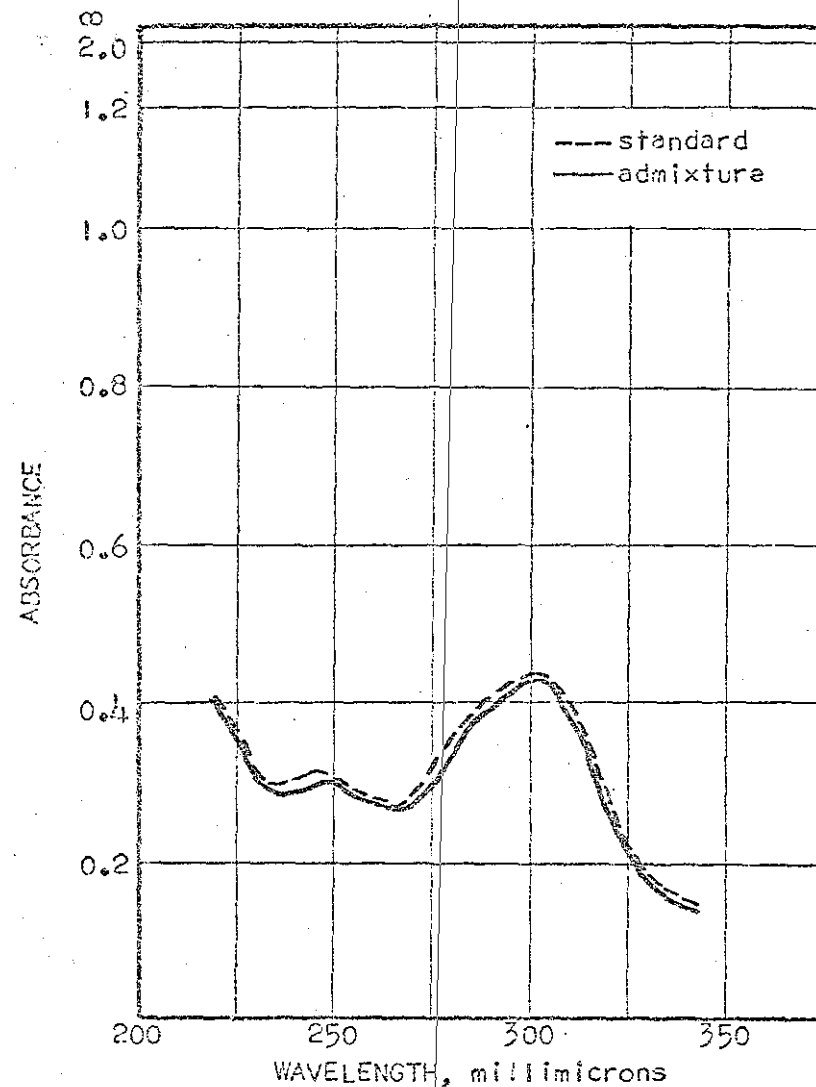


Methotrexate Sodium 8 mcg./ml.
Ref: Sodium Cephalothin 20 mcg./ml.

Figure 28. U.V. Spectrum of Sodium Cephalothin (λ_{max} 236 mμ) with Methotrexate Sodium (λ_{max} 302 mμ) in 5 percent Dextrose Injection at one hour.

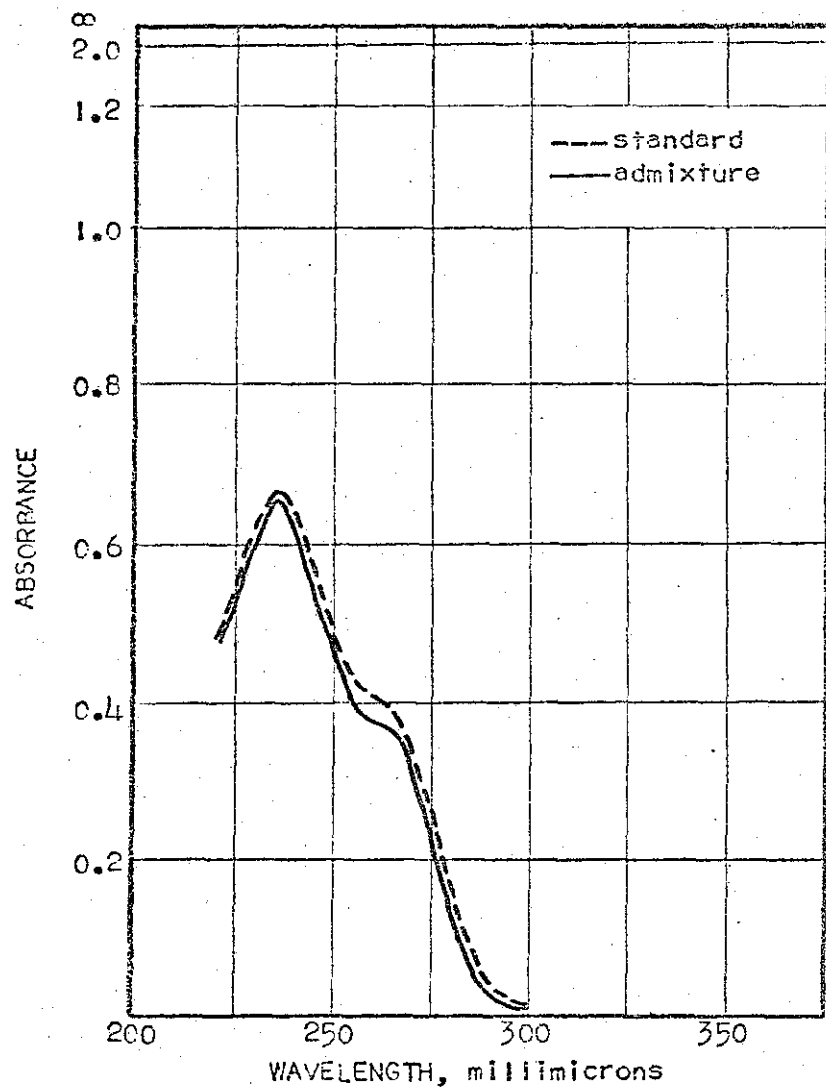


Sodium Cephalothin 20 mcg./ml.
Ref: Methotrexate Sodium 8 mcg./ml.

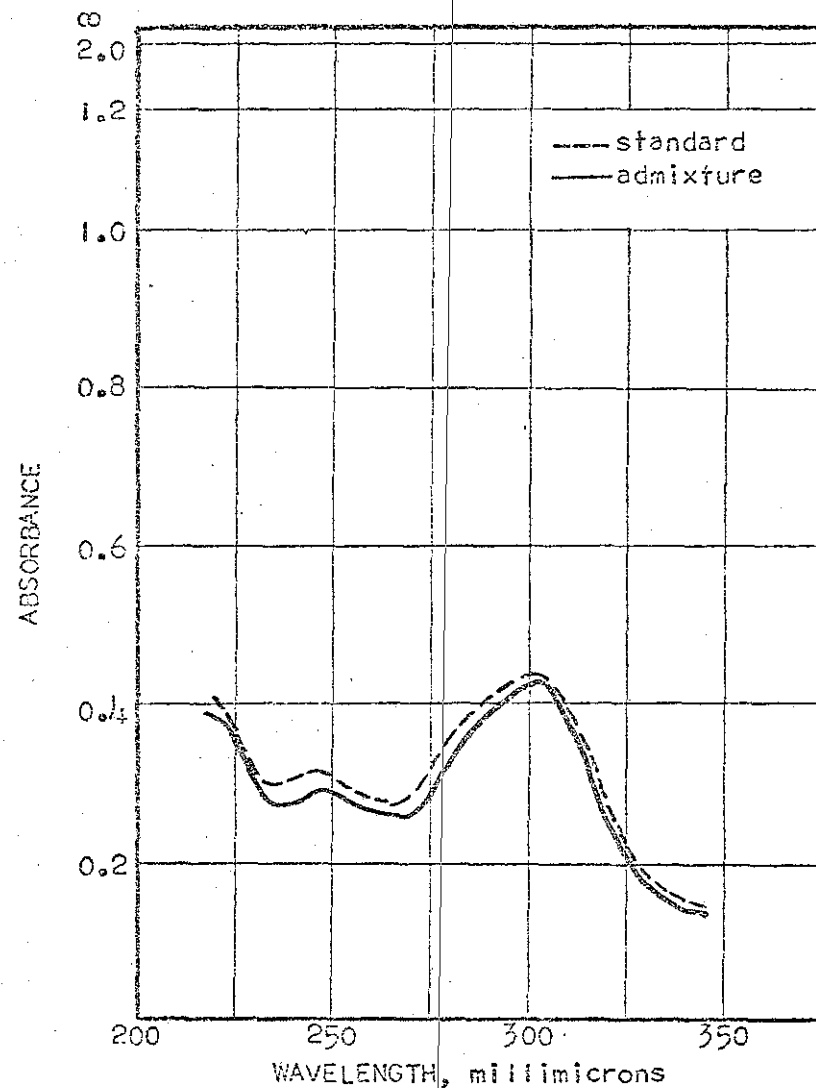


Methotrexate Sodium 8 mcg./ml.
Ref: Sodium Cephalothin 20 mcg./ml.

Figure 29. U.V. Spectrum of Sodium Cephalothin (λ_{\max} 236 mμ) with Methotrexate Sodium (λ_{\max} 302 mμ) in 5 percent Dextrose Injection at four hours.



Sodium Cephalothin 20 mcg./ml.
Ref: Methotrexate Sodium 8 mcg./ml.



Methotrexate Sodium 8 mcg./ml.
Ref: Sodium Cephalothin 20 mcg./ml.

Figure 30. U.V. Spectrum of Sodium Cephalothin (λ_{\max} 236 mμ) with Methotrexate Sodium (λ_{\max} 302 mμ) in 5 percent Dextrose Injection at eight hours.

ADMIXTURE #11 METHOTREXATE---VINCRIStINE SULFATE

A special problem was encountered in preparing an admixture of methotrexate sodium and vincristine sulfate because of the extremely small doses of vincristine sulfate normally employed in clinical medicine. For this particular additive, the usual recommended therapeutic concentration was also the optimum concentration for spectrophotometric scanning.

In order for both drugs to be mixed together and still obtain optimum spectrophotometric concentrations for each drug, different dilution techniques were employed. A single component admixture of methotrexate sodium in 5 percent Dextrose Injection was prepared having a concentration of 200 mcg./ml.

Vincristine sulfate was reconstituted with the accompanying diluent according to the manufacturer's recommendations. A dilution of vincristine sulfate was prepared to yield a concentration of 4 mcg./ml. To this solution an aliquot amount of the methotrexate sodium solution was added to produce a concentration of 8 mcg./ml. in the admixture. While this technique did not simulate actual clinical practice, this method was employed nevertheless with the hope that useful compatibility information could be obtained.

The concentration used for vincristine sulfate was calculated on the following basis for a 70 Kg. person receiving the drug over a 24 hour period in three divided doses, each of which to be delivered in 500 ml. of the intravenous

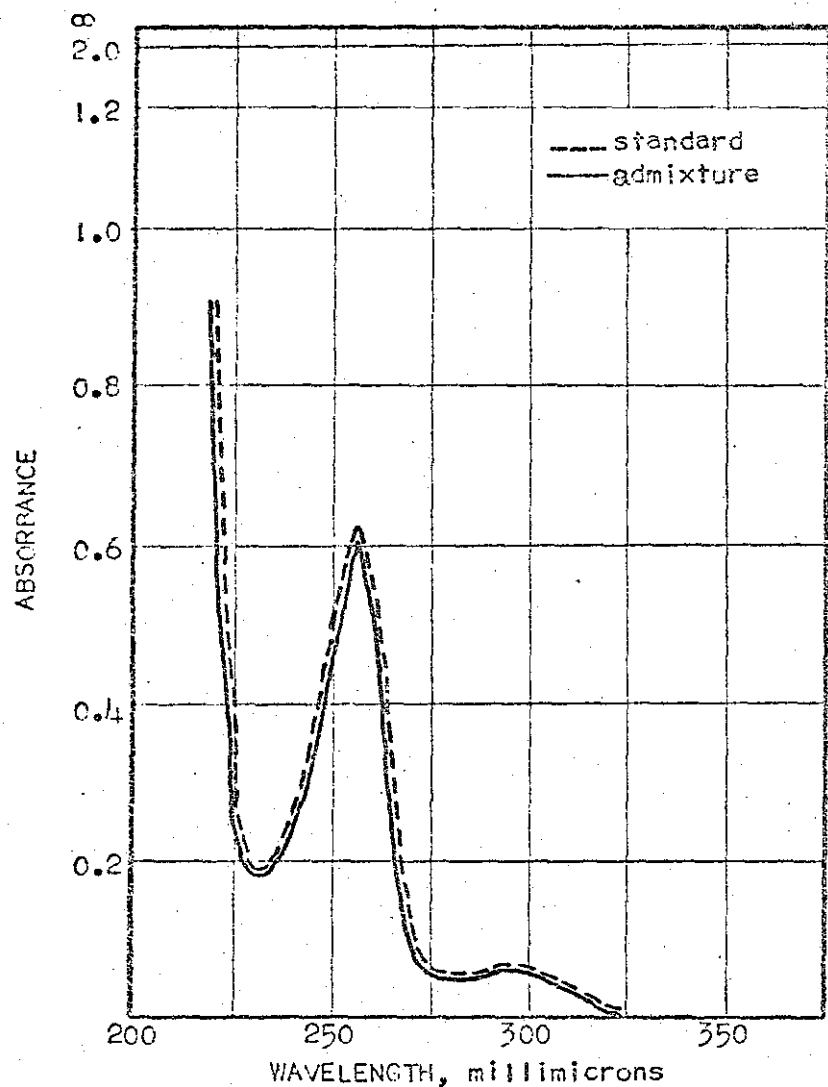
fluid:

Vincristine Sulfate

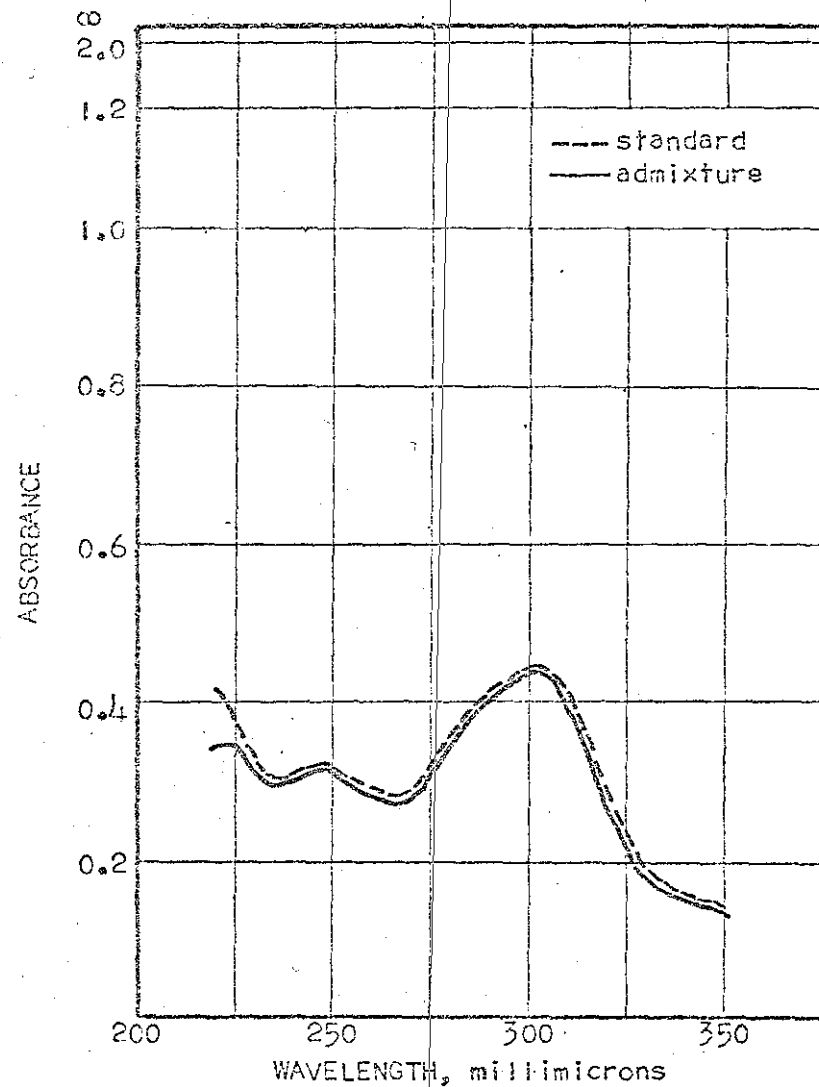
Usual Recommended Dose	0.05 mg./Kg.-0.1 mg./Kg. ^a
Dose for 70 Kg. Adult	3.5 mg./Kg./Day-7.0 mg./Kg./Day
Dose for 8 Hrs.	1.17 mg.-2.33 mg.
Therapeutic Conc. Used	4 mcg./ml.

The results of the analysis of the spectrum obtained for each of the drugs in the admixture indicated that there did not appear to be any alteration in either shape or any decrease in absorbance. (Figures 31, 32, 33)

a - Product Information, Eli Lilly, Indianapolis, Ind.

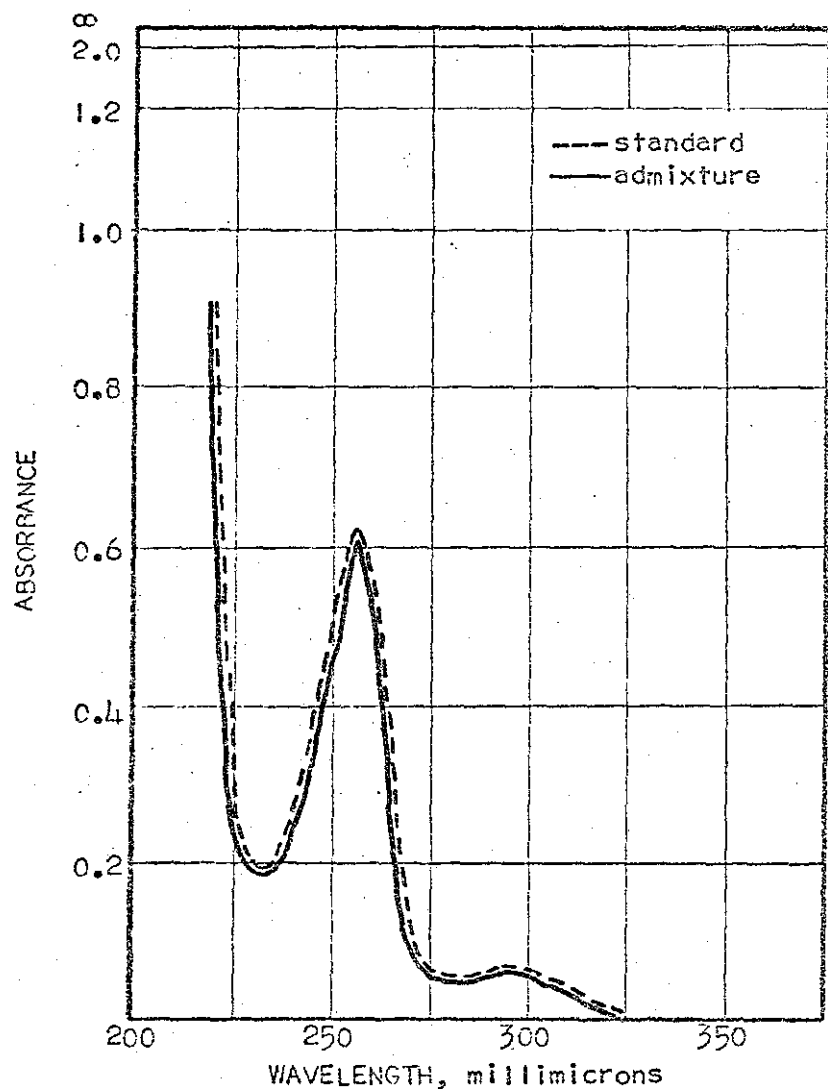


Vincristine Sulfate 4 mcg./ml.
Ref: Methotrexate Sodium 8 mcg./ml.

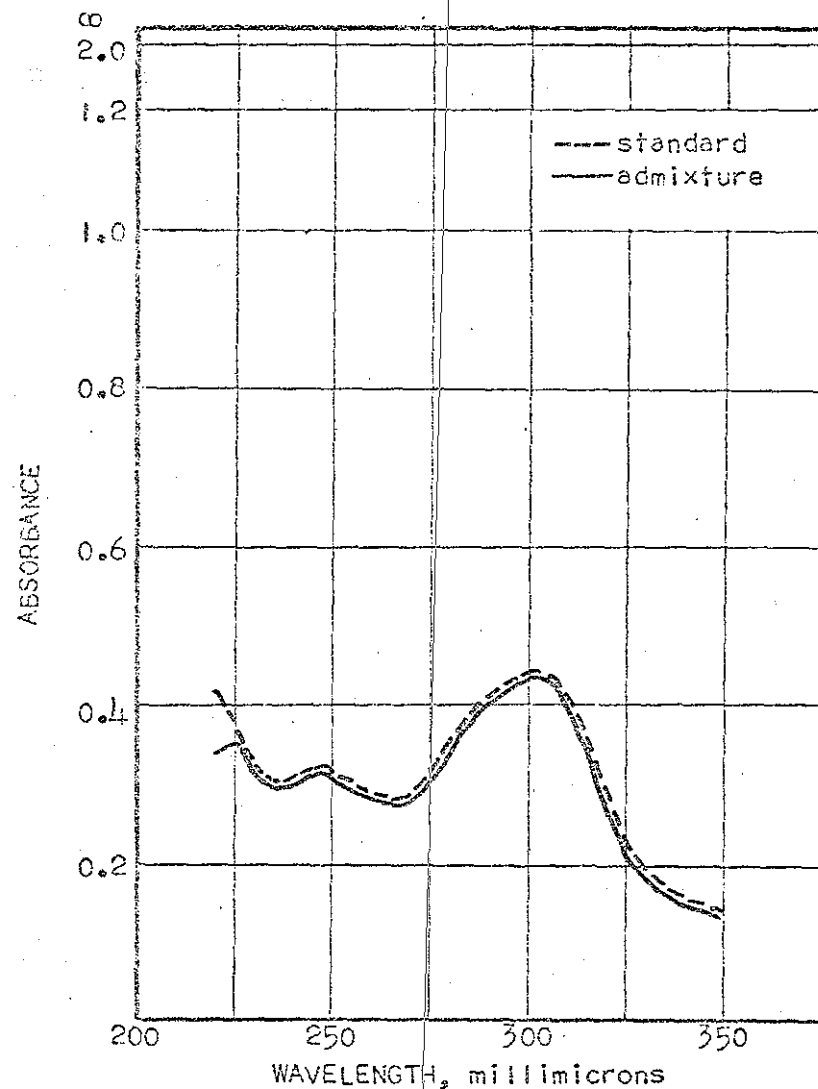


Methotrexate Sodium 8 mcg./ml.
Ref: Vincristine Sulfate 4 mcg./ml.

Figure 31. U.V. Spectrum of Vincristine Sulfate (λ_{\max} 256.5 mμ) with Methotrexate Sodium (λ_{\max} 302 mμ) in 5 percent Dextrose Injection at one hour.

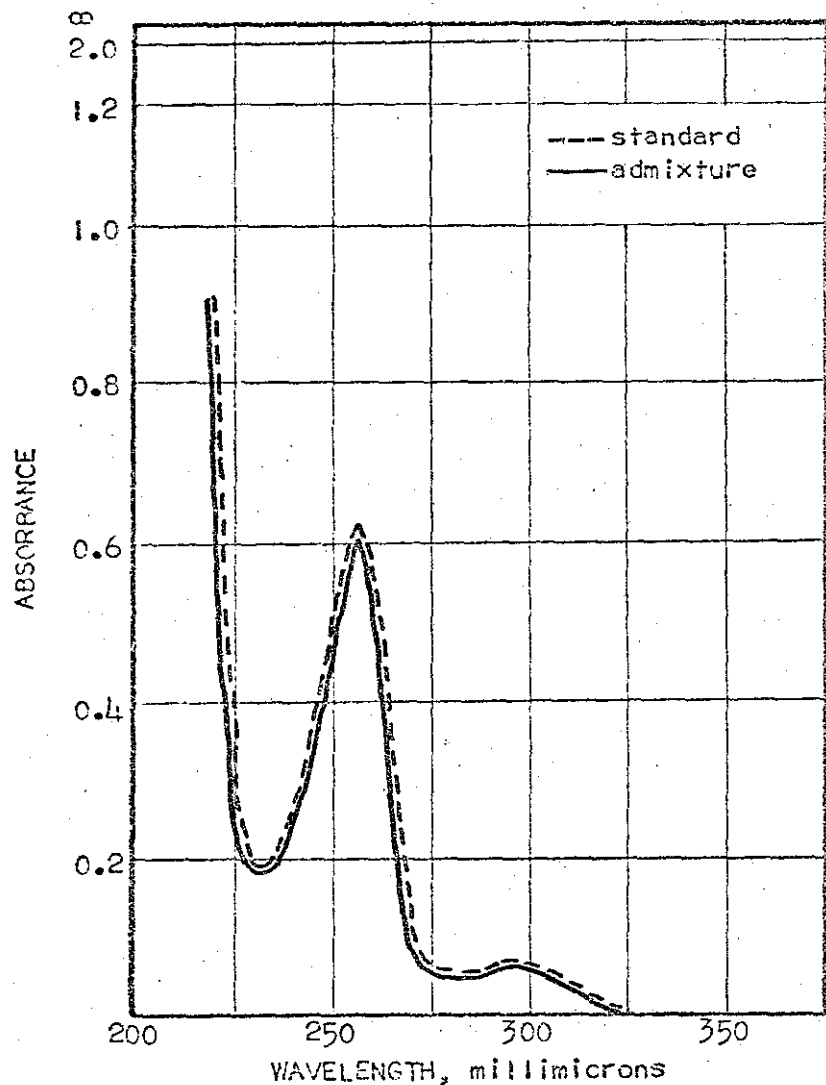


Vincristine Sulfate 4 mcg./ml.
Ref: Methotrexate Sodium 8 mcg./ml.

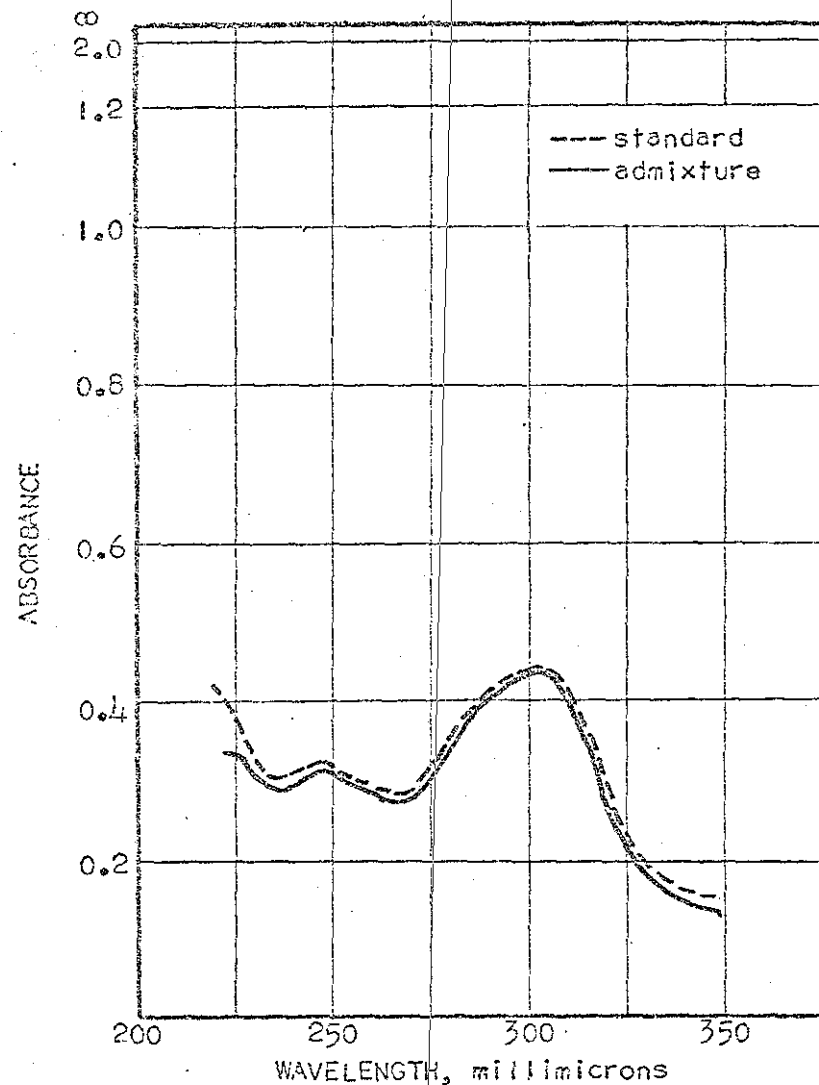


Methotrexate Sodium 8 mcg./ml.
Ref: Vincristine Sulfate 4 mcg./ml.

Figure 32. U.V. Spectrum of Vincristine Sulfate (λ_{\max} 256.5 mμ) with Methotrexate Sodium (λ_{\max} 302 mμ) in 5 percent Dextrose Injection at four hours.



Vincristine Sulfate 4 mcg./ml.
Ref: Methotrexate Sodium 8 mcg./ml.



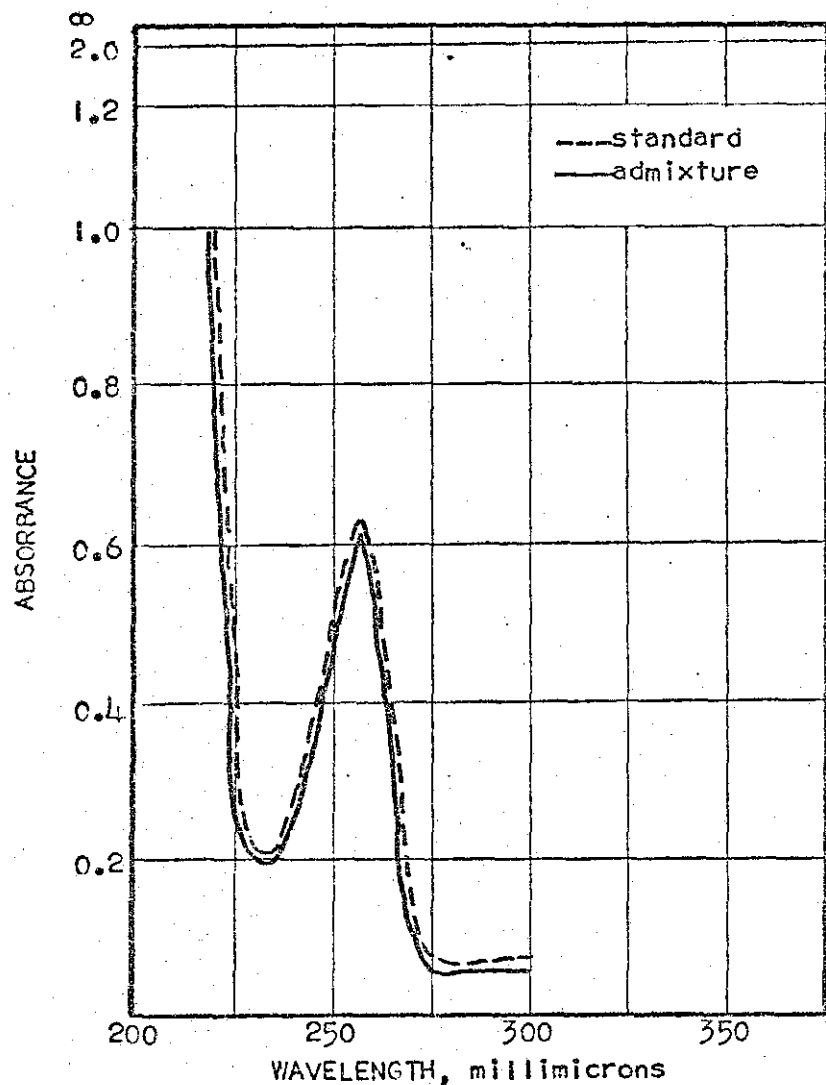
Methotrexate Sodium 8 mcg./ml.
Ref: Vincristine Sulfate 4 mcg./ml.

Figure 33. U.V. Spectrum of Vincristine Sulfate (λ_{\max} 256.5 mμ) with Methotrexate Sodium (λ_{\max} 302 mμ) in 5 percent Dextrose Injection at eight hours.

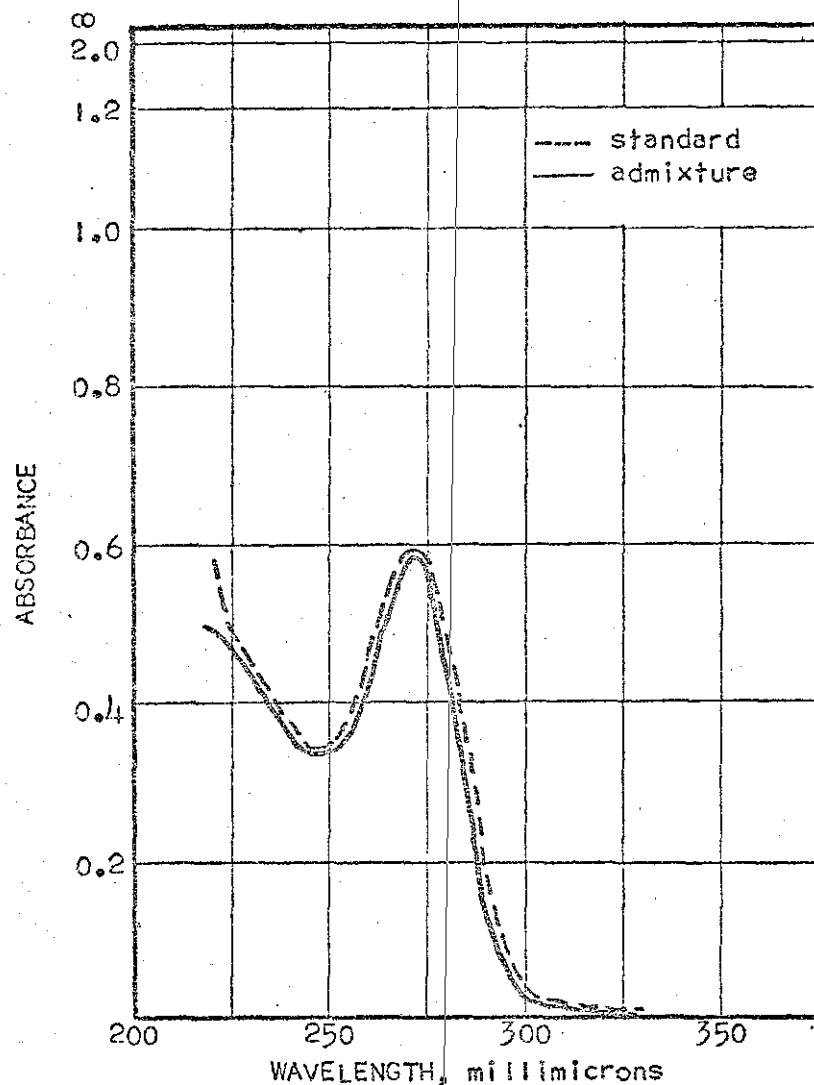
ADMIXTURE #12 CYTARABINE--VINCRISTINE SULFATE

In this admixture, cytarabine was added to vincristine sulfate. As with the previous admixture, dilution procedures were somewhat modified. A single component admixture of cytarabine in the infusion fluid was prepared which had a resulting concentration of 400 mcg./ml. After reconstitution, a solution of vincristine sulfate was made with a resulting concentration of 4 mcg./ml. To the vincristine sulfate solution was added an aliquot portion of the cytarabine solution to produce a final concentration of cytarabine of 16 mcg./ml. in the admixture. The solution was then scanned and the spectrum for each drug was analyzed for any sign of a chemical incompatibility.

For each of the two drugs in the admixture, the absorption spectrum was compared with its respective reference standard. Since, throughout the eight hour study, the ultraviolet absorption spectrum for the two drugs appeared not to be altered, it would appear that the two drugs are chemically compatible in admixture. (Figures 34, 35, 36)

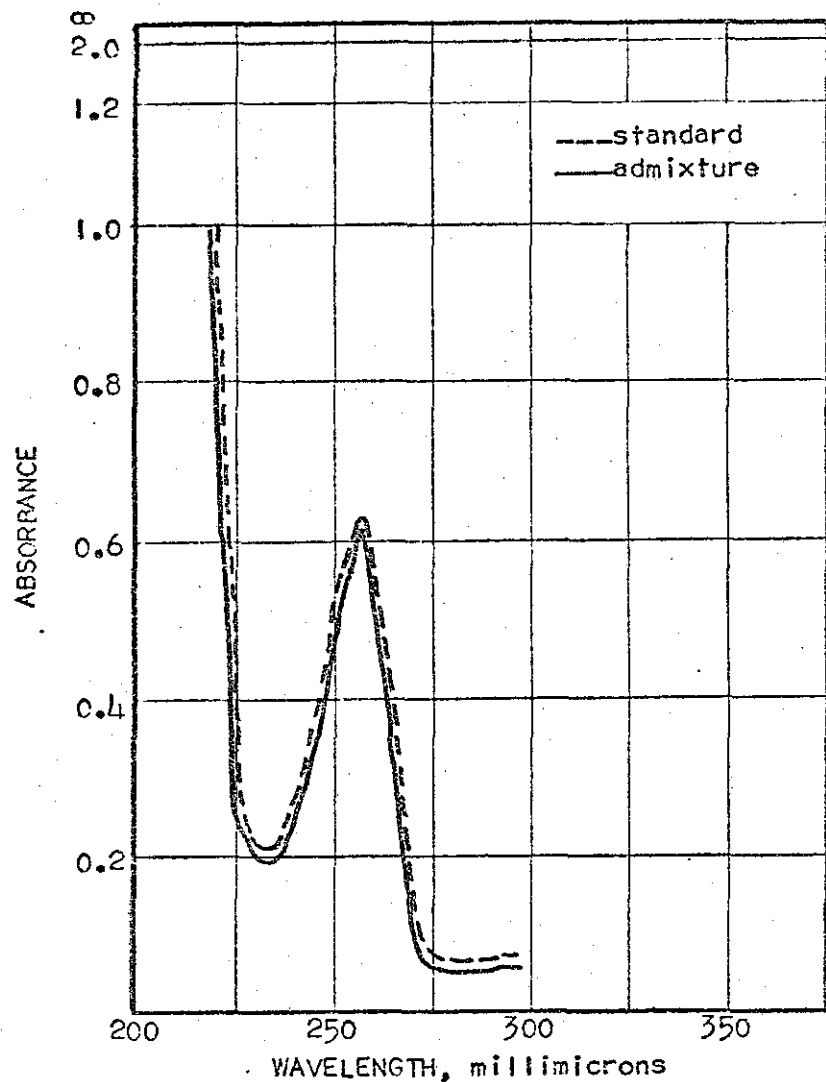


Vincristine Sulfate 4 mcg./ml.
Ref: Cytarabine 16 mcg./ml.

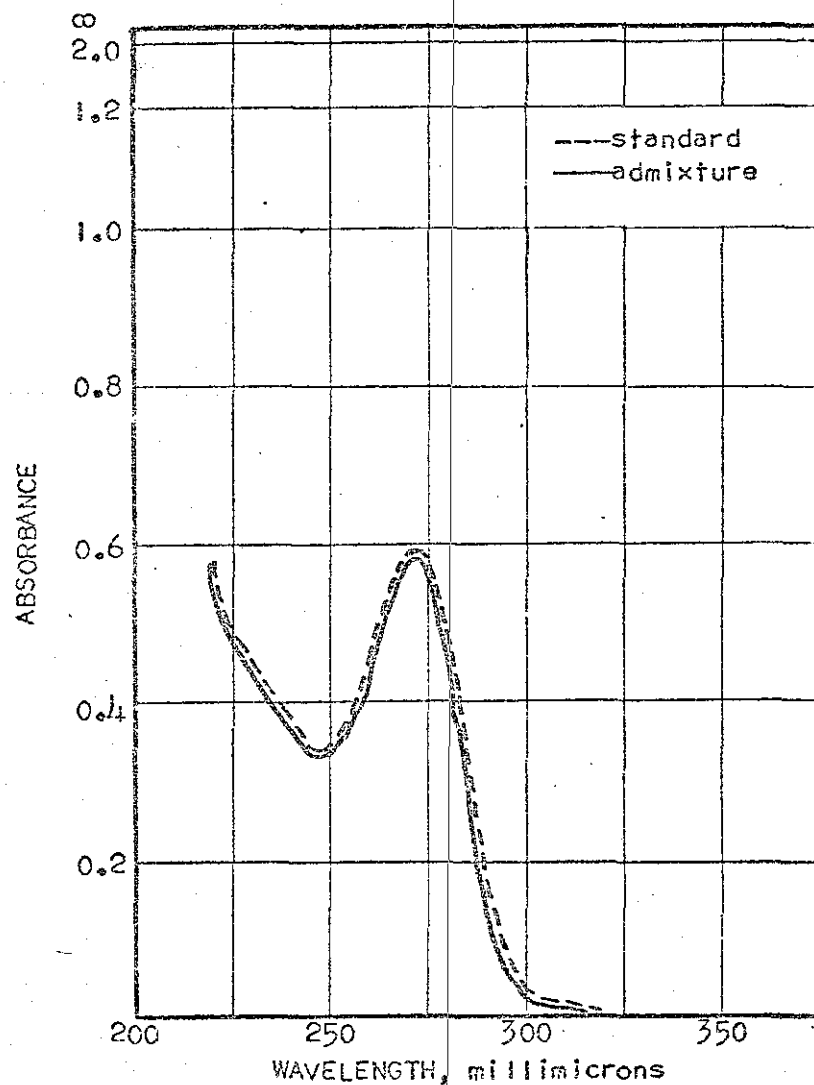


Cytarabine 16 mcg./ml.
Ref: Vincristine Sulfate 4 mcg./ml.

Figure 34. U.V. Spectrum of Vincristine Sulfate (λ_{\max} 256.5 mμ) with Cytarabine (λ_{\max} 272 mμ) in 5 percent Dextrose Injection at one hour.

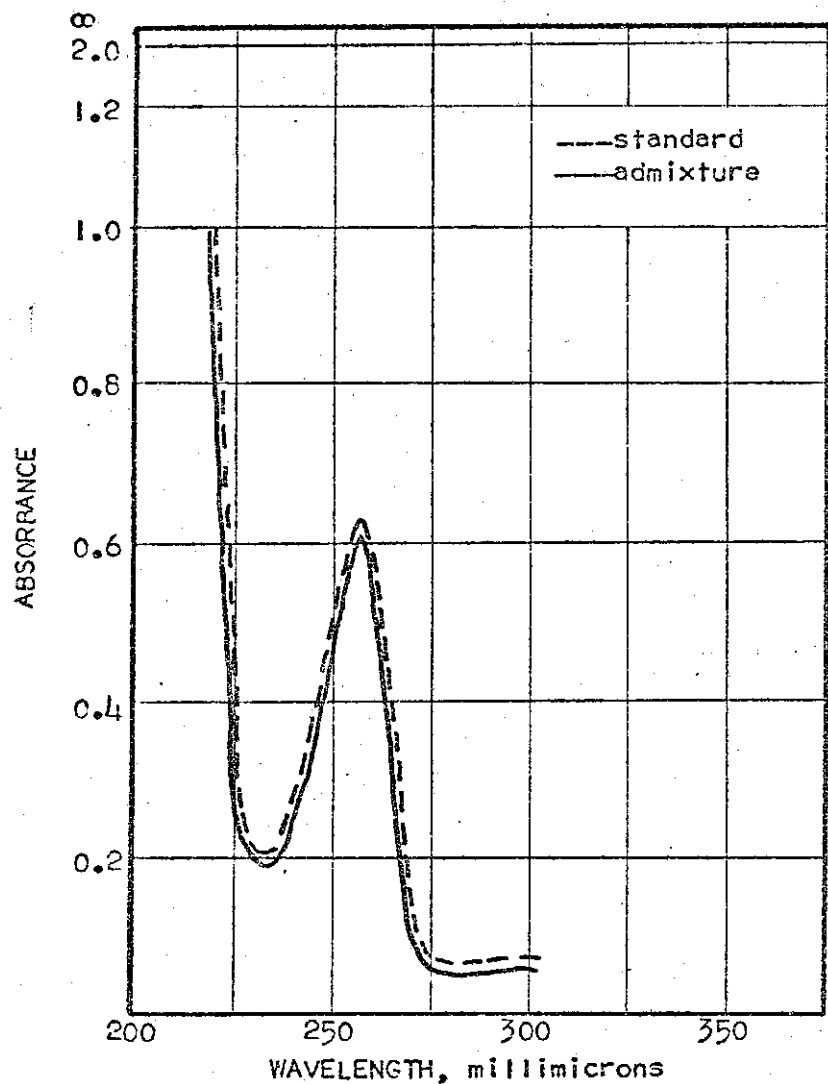


Vincristine Sulfate 4 mcg./ml.
Ref: Cytarabine 16 mcg./ml.

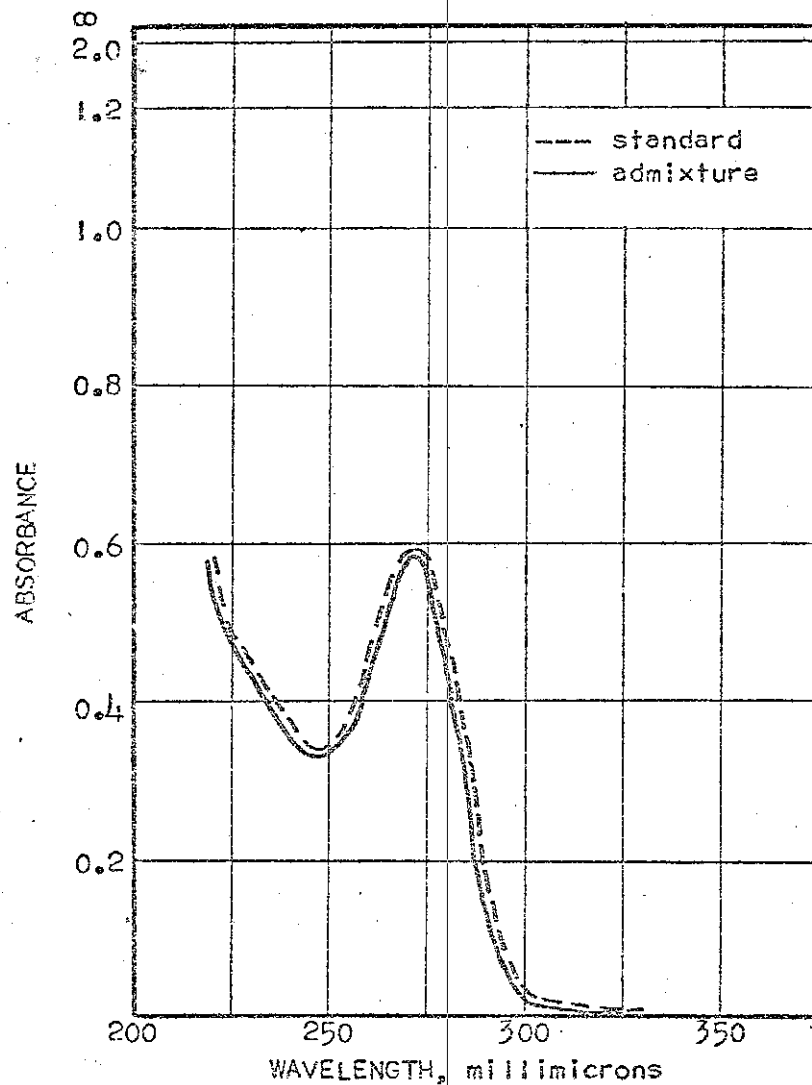


Cytarabine 16 mcg./ml.
Ref: Vincristine Sulfate 4 mcg./ml.

Figure 35. U.V. Spectrum of Vincristine Sulfate (λ_{\max} 256.5 mμ) with Cytarabine (λ_{\max} 272 mμ) in 5 percent Dextrose Injection at four hours.



Vincristine Sulfate 4 mcg./ml.
Ref: Cytarabine 16 mcg./ml.



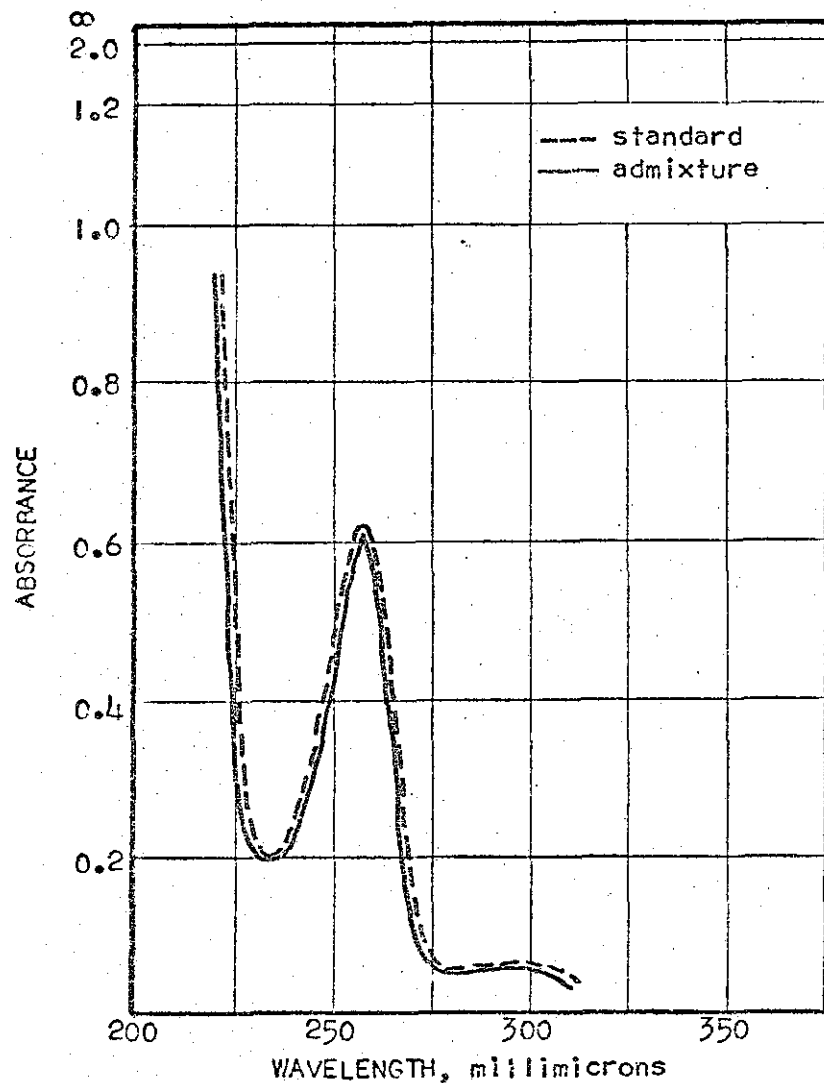
Cytarabine 16 mcg./ml.
Ref: Vincristine Sulfate 4 mcg./ml.

Figure 36. U.V. Spectrum of Vincristine Sulfate (λ_{max} 256.5 mμ) with Cytarabine (λ_{max} 272 mμ) in 5 percent Dextrose Injection at eight hours.

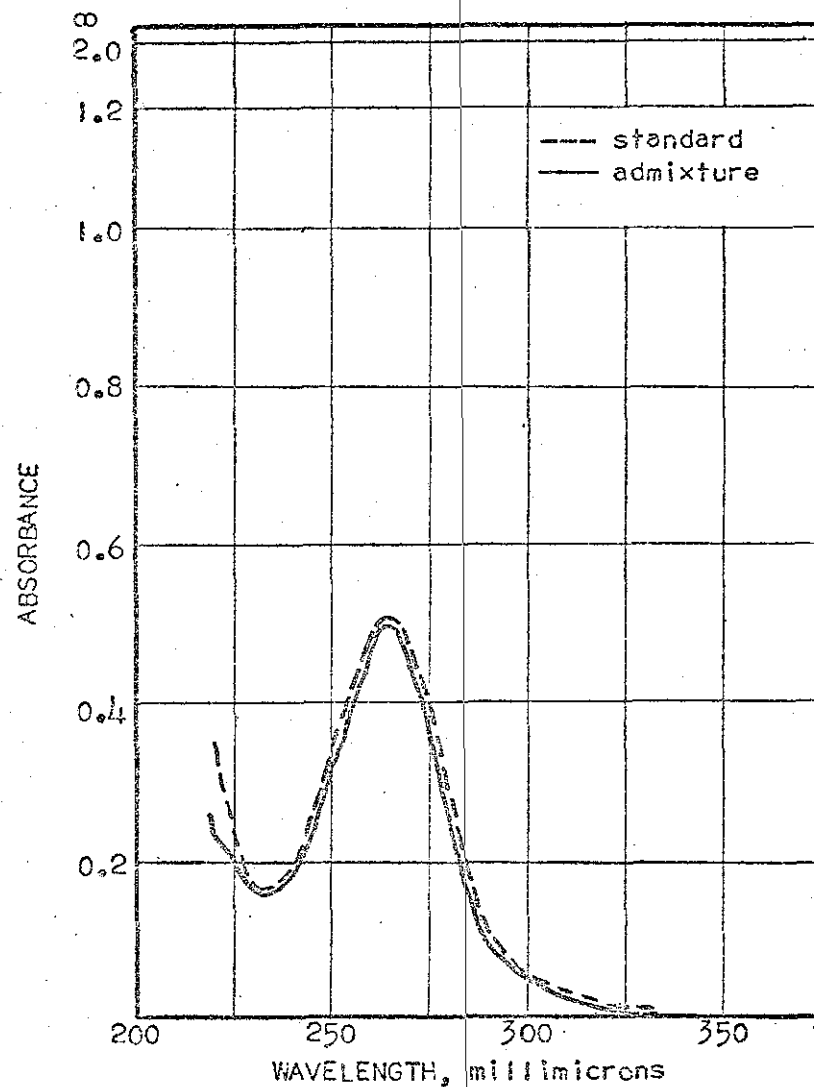
ADMIXTURE #13 5-FLUOROURACIL--VINCRISTINE SULFATE

In the last of the admixtures done in this study, 5-fluorouracil was added to vincristine sulfate. As was mentioned before, the admixture techniques when using vincristine sulfate were modified. A single component admixture of 5-fluorouracil in the 5 percent Dextrose Injection was made to give a concentration of 500 mcg./ml. A solution of vincristine sulfate was made with a concentration of 4 mcg./ml. To this latter solution, an aliquot amount of the 5-fluorouracil solution was added to give a concentration of 10 mcg./ml. in the admixture. A spectrum for each of the two drugs in the admixture was then obtained with the spectrophotometer.

Although the absorbance of the 5-fluorouracil decreased slightly over the eight hour period, the decrease was not significant and the spectrum for 5-fluorouracil remained essentially unchanged. The absorbance and the spectrum for the vincristine sulfate remained unchanged through the study. This would tend to indicate that there is no chemical incompatibility when 5-fluorouracil is mixed with vincristine sulfate. (Figures 37, 38, 39)

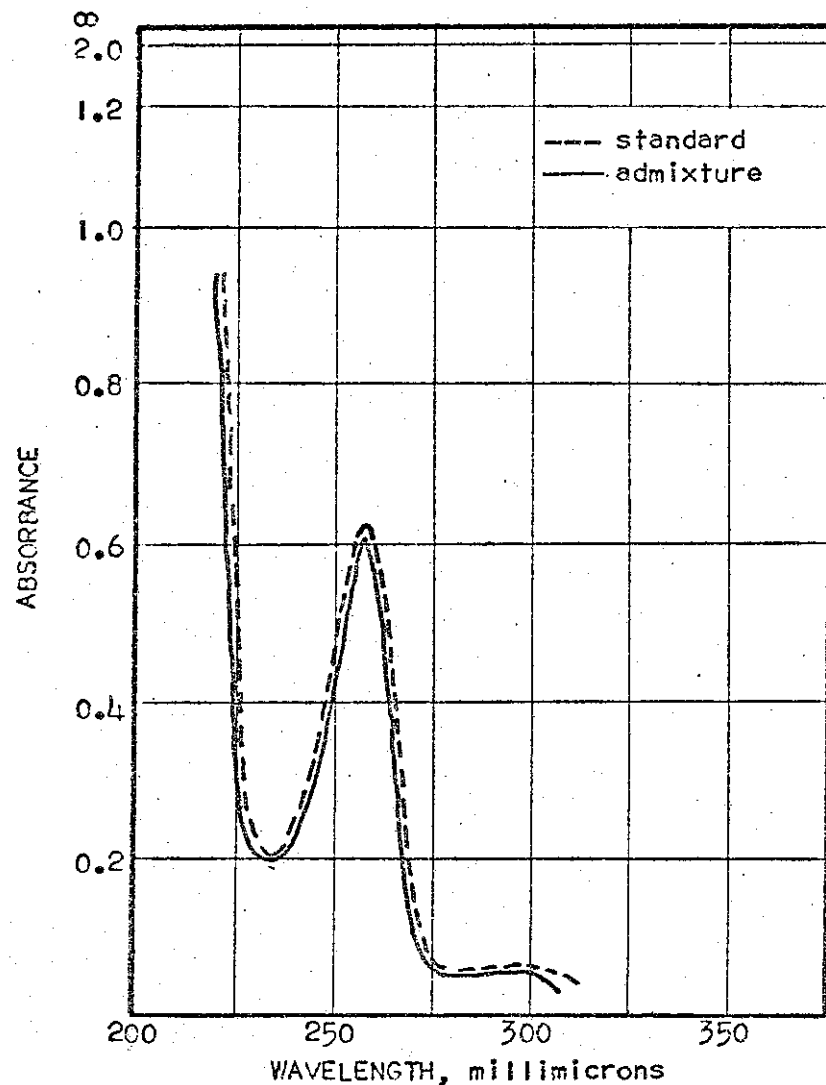


Vincristine Sulfate 4 mcg./ml.
Ref: 5-Fluorouracil 10 mcg./ml.

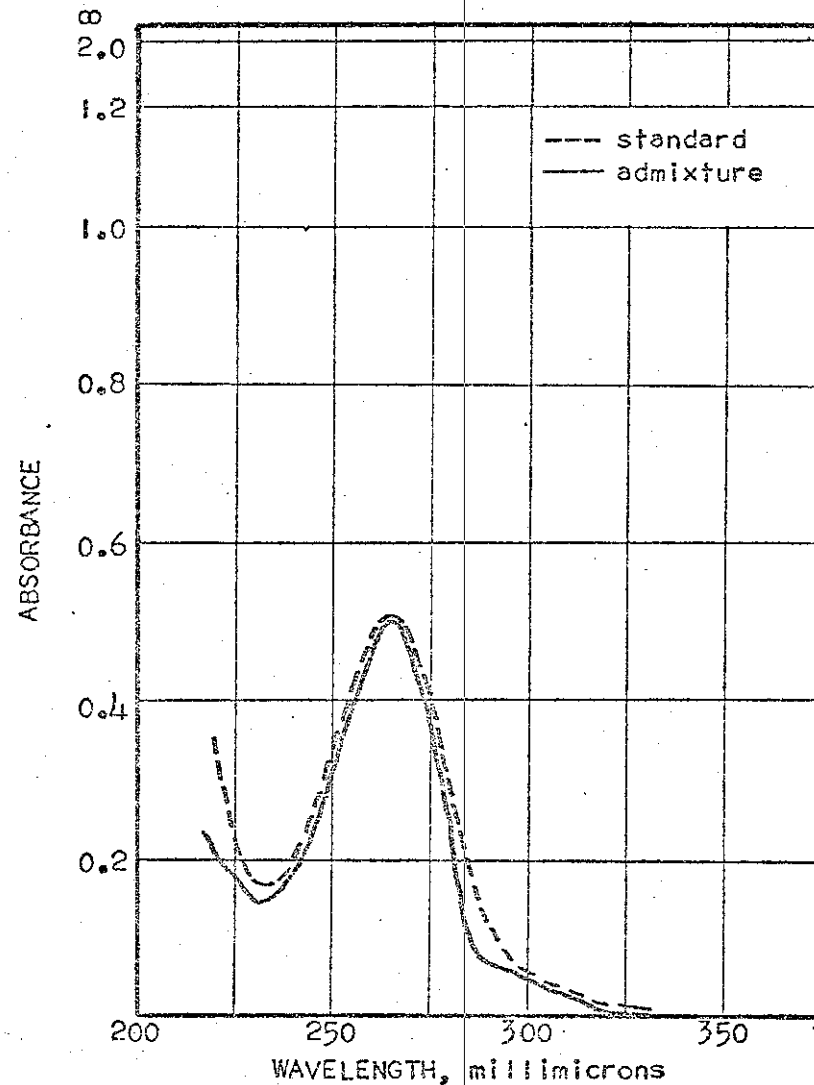


5-Fluorouracil 10 mcg./ml.
Ref: Vincristine Sulfate 4 mcg./ml.

Figure 37. U.V. Spectrum of Vincristine Sulfate (λ_{max} 256.5 mμ) with 5-Fluorouracil (λ_{max} 265.5 mμ) in 5 percent Dextrose Injection at one hour.

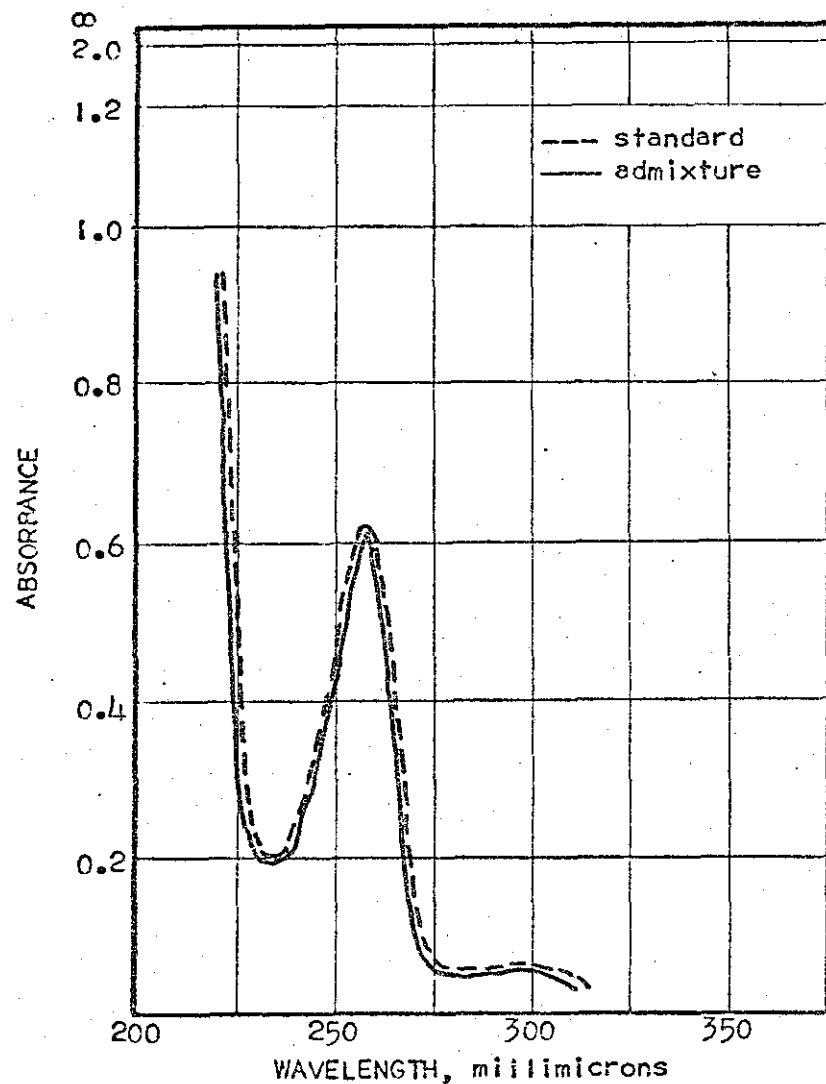


Vincristine Sulfate 4 mcg./ml.
Ref: 5-Fluorouracil 10 mcg./ml.

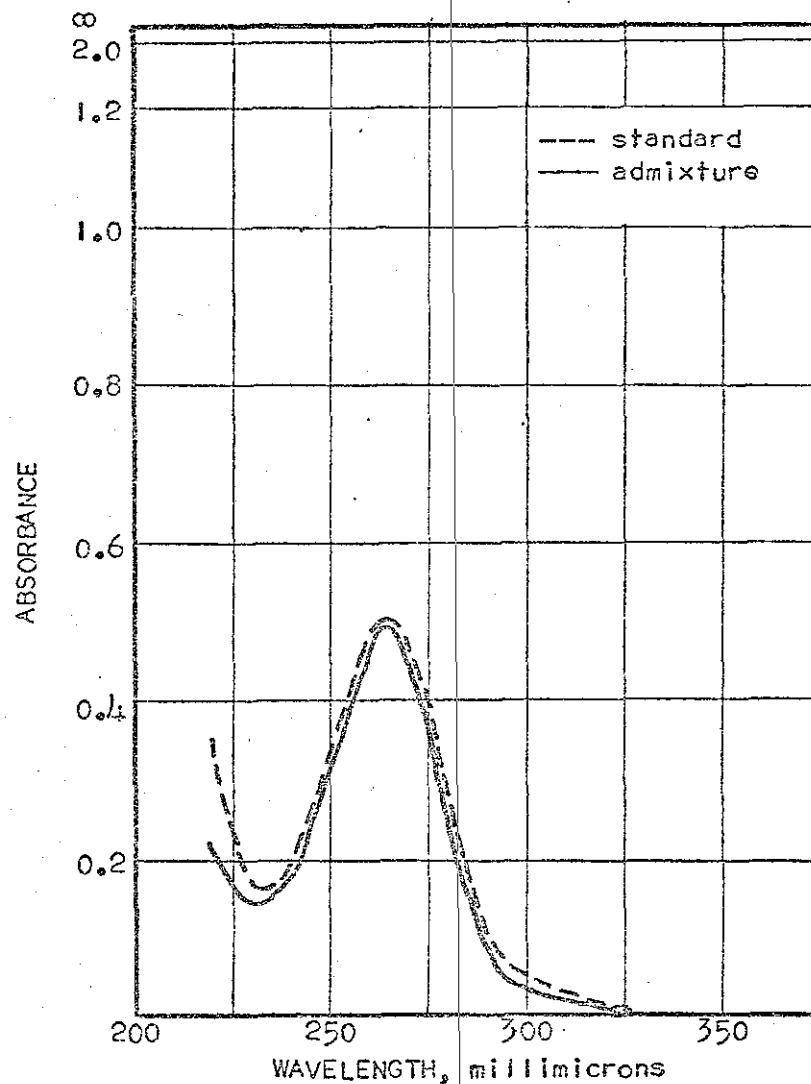


5-Fluorouracil 10 mcg./ml.
Ref: Vincristine Sulfate 4 mcg./ml.

Figure 38. U.V. Spectrum of Vincristine Sulfate (max 256.5 mμ) with 5-Fluorouracil (max 265.5 mμ) in 5 percent Dextrose Injection at four hours.



Vincristine Sulfate 4 mcg./ml.
Ref: 5-Fluorouracil 10 mcg./ml.



5-Fluorouracil 10 mcg./ml.
Ref: Vincristine Sulfate 4 mcg./ml.

Figure 39. U.V. Spectrum of Vincristine Sulfate (λ_{\max} 256.5 mμ) with 5-Fluorouracil (λ_{\max} 265.5 mμ) in 5 percent Dextrose Injection at eight hours.

TABLE III (CONTINUED)

Admixtures ^a	Concentration (mcg./ml.) ^c		Max ^d	Spectrum ^{ef}
	Therapeutic	Dilution Scanned		
11. Methotrexate Sodium	8	8	302	no change
Vincristine Sulfate	4	4	256.5	no change
12. Cytarabine	16	16	272	no change
Vincristine Sulfate	4	4	256.5	no change
13. 5-Fluorouracil	10	10	265.5	no change
Vincristine Sulfate	4	4	256.5	no change

- a - In the following admixtures, the first drug listed in the combination pair was added to the second drug listed.
- b - Higher therapeutic concentrations of these drugs were used when mixed with sodium cephalothin in order that both drugs in the admixture could be spectrophotometrically measured after proper dilution.
- c - Dilutions of the drugs from their therapeutic concentrations were necessary in order to achieve concentrations that could be spectrophotometrically measured.
- d - The values listed for the λ_{max} for each of the drugs are those that were obtained by measuring each drug alone in Dextrose 5 percent Injection.
- e - The spectrum for each of the drugs in the combinations was considered altered if there was a significant loss (greater than 10 percent) in absorption of the drug or if there was an appreciable change in the shape or structure of the absorption spectrum.
- f - No physical or visual incompatibilities were noticed in any of the admixtures that might have been determined by a color change, precipitation, cloudiness, or gas evolution.

CHAPTER IV

DISCUSSION

In reviewing the results of this experiment, it is apparent that there may be chemical incompatibilities among certain of the oncolytic agents tested when mixed together with themselves or with cephalothin sodium or prednisolone sodium phosphate in intravenous solution, using 5 percent Dextrose Injection as the infusion vehicle.

The findings demonstrated definite spectral alterations of both drugs when 5-fluorouracil was added to methotrexate sodium. The changes in the spectra of the drugs in this admixture were the most noticeable of any of the admixtures throughout the entire study. The increase in the absorbance of 5-fluorouracil was quite apparent, however, the clinical significance of this is not clear. It would appear that the change is related to a chemical change in the chemical structure of the drug. The changes in the spectrum of methotrexate sodium were quite startling. Chemical alterations in the structure of the drug are probably the cause of the changes that were noted in the spectrum of methotrexate sodium when it was combined with the 5-fluorouracil. On the basis of these results, it is advised that this combination of drugs not be mixed together in an

I.V. bottle.

A change was observed in the spectrum of prednisolone sodium phosphate when it was added to methotrexate sodium. The spectrum of methotrexate itself was altered to a lesser extent. Therefore, in the interest of the chemical integrity of both of these drugs, use of this combination should be approached with caution.

While only slight spectral changes were noted in methotrexate sodium when it was added to cytarabine, it is important to keep in mind that while these alterations appeared to be only relatively small, significant chemical and hence, possibly significant therapeutic changes might have occurred.

In the admixture in which 5-fluorouracil was added to cytarabine, no alteration was observed in the spectrum for the 5-fluorouracil component. However, there was a somewhat noticeable change in the spectrum for the cytarabine. Again, the compatibility of this combination in admixture may be open to some question, and if possible, the mixing of these drugs in an I.V. bottle should be discouraged.

It is of interest to note that in those admixtures in which vincristine sulfate and sodium cephalothin were included no significant or apparent alterations in spectra were noted. Also, when prednisolone sodium phosphate was added to 5-fluorouracil and to cytarabine, no chemical

interactions or incompatibilities were noticed. From the results of this study, it appears that the combining of these drugs in 5 percent Dextrose Injection in admixtures does not result in any "visual" or "physical" or chemical incompatibilities.

It was mentioned earlier that little work had previously been done in the area of intravenous incompatibilities involving anticancer drugs. One reference, mentioned earlier, is applicable to the results of this study. In the compatibility table which was compiled by the Pharmacy Department at the National Institutes of Health, it is stated that methotrexate sodium is compatible with vincristine sulfate (74). The work done in this study would further verify that statement. It is unfortunate that in their compatibility table, no mention was made to indicate how compatibility was determined.

Although not found in the literature, personal correspondence of the author with the Pharmacy Department at N.I.H. indicated that the admixture of methotrexate sodium and prednisolone sodium phosphate was incompatible. Again, the results of this experiment, in Admixture #6, would tend to confirm that fact.

Out of the four admixtures in which some spectral change was observed, it is of interest to note that methotrexate sodium was involved in three, and 5-fluorouracil, prednisolone sodium phosphate, and cytarabine in one admixture. Two of the drugs in this study, as mentioned above,

did not appear to be involved in any chemical or physical incompatibility.

Because methotrexate sodium was the drug most frequently involved in the admixtures whose additives showed altered spectra, and because of the significant changes that were noted when 5-fluorouracil was added to methotrexate sodium, it was decided to examine this particular combination of drugs more closely. This was in an attempt to further demonstrate and validate that an incompatibility does exist when methotrexate sodium and 5-fluorouracil are mixed together in an intravenous solution. It was hoped that by employing a different method of detecting chemical interactions, such as through the demonstration of an altered or breakdown product, that the results of the spectrophotometric analysis would be confirmed and firmly substantiated. Since, in Admixture #2, the spectrum of methotrexate sodium demonstrated the greatest alteration, it was decided to examine this drug, alone and in the combination with the 5-fluorouracil to further establish that a chemical change had occurred.

Only a few other examples of other possible and practical methods of examining methotrexate sodium in an admixture were found in the literature. Most of these involved elaborate procedures employing complex chemical techniques such as fluorometry (76), microbiological assays (77,78), spectrofluorometry (79), and chromatography (80,81), as well as the U.S.P. method of ultraviolet

spectrophotometry (82). These papers describing methods of column and paper chromatography expressed several limitations to such techniques. Therefore, a novel technique was attempted utilizing thin-layer chromatography because of its suitability, relatively easy application, and wide usage in other assay procedures (83,84).

Since no previous assay method for methotrexate sodium (or for 5-fluorouracil) using thin-layer chromatography could be found in the literature, techniques and materials were devised on an empirical basis. Using a variety of solvent systems on three different adsorbants, attempts were made to find a combination which would result in different R_f values or chromatograms for methotrexate sodium and 5-fluorouracil. It was hoped that the two compounds could be distinguished and identified.

Eluotropic systems for this phase of study included solvents with various degrees of polarity based on available tables that provided a breakdown of the eluting power of certain solvents (85). Also, included were eluotropic systems with different degrees of acidity and alkalinity. Combinations of solvents were selected on the basis of predicted utility for these drugs. Trial adsorbants included activated silics gel, activated alumina, and cellulose. These were tested in order to experiment with adsorbants of different strengths of adsorbing power.

Thin-layer chromatography was applied to methotrexate sodium and 5-fluorouracil in solutions by themselves as well

as an admixture of the two drugs. After spotting the plates and allowing the solvent systems to ascend 10 cm., the plates were allowed to dry before being developed with a spray of either potassium permanganate 0.05 percent in water or a mixture of potassium permanganate 1 percent and sodium carbonate 5 percent in water. After extensive attempts of testing, including attempts at two-dimensional thin-layer chromatography, no combination of solvent system and adsorbant could be found that would adequately separate and, therefore, distinguish the two drugs, alone or in the admixture.

Approaching the incompatibility problem of methotrexate sodium and 5-fluorouracil from a different point of view, another method was sought that would substantiate the results of the spectrophotometric analysis. It was reasoned that if an alteration in the spectrum of the two drugs resulted only from the change in the pH of the admixture, the original spectrum should again be attained by simply readjusting the pH of the admixture back to the original pH of the reference solutions of each drug. If the original spectra could not be attained by this method, then it could be concluded that the alterations that occurred in the spectra were of a permanent nature indicating a change in the chemical nature of the two drugs.

In an attempt to test this theory, the admixture (see Admixture #2) was repeated, including preparation of

the reference solutions. The pH of each of the solutions was measured using a Radiometer pH Meter 25 SE Expanded Scale^a model to first see if there existed any differences in the pH of the reference solutions of each of the drugs and the admixture solution. The results of the pH readings are shown on the following table.

TABLE IV

Change in pH When 5-Fluorouracil Was Added to
Methotrexate Sodium in 5 Percent Dextrose Injection

Drug	Concentration (mcg./ml.)	pH
Methotrexate Sodium	200	6.87
5-Fluorouracil	250	8.51
5 Percent Dextrose Injection		4.5
Admixture (Therapeutic Conc.)		
Methotrexate Sodium	200	8.4
5-Fluorouracil	250	
Admixture (Scanning Dilution)		
Methotrexate Sodium	8	7.2
5-Fluorouracil	10	

From the data above, it is seen that there were indeed pH changes in the admixed solutions. It was thought that there might be some possible correlation between the results

a - Radiometer, Copenhagen, Denmark.

obtained with the use of the spectrophotometer and the changes in pH. It is of interest to note that the pH of the admixture was very close to the pH of the 5-fluorouracil reference solution and it was quite different from the original pH of the methotrexate sodium reference solution indicating that the buffer capacity of the 5-fluorouracil was stronger than that of the methotrexate sodium.

In the next step of the attempt to correlate changes in pH with the alteration in the spectrum of methotrexate sodium, a solution of methotrexate sodium was made in a buffer solution whose pH approximated the pH of the admixture. This buffer solution was an alkaline borate buffer of pH 8.2 (76). The concentration of methotrexate sodium used was the same as before, 200 mcg./ml., and the pH of the resulting solution was pH 7.85. It was thought that if the same altered spectra were obtained in the buffered solution, the spectral alteration would probably be due to the change in pH. After scanning this solution with the spectrophotometer, the same altered spectrum of methotrexate sodium was obtained as occurred when in the admixture with 5-fluorouracil.

To carry this experiment out one step further, the pH of the buffered solution of methotrexate sodium was lowered in a step-wise manner, using 0.2M HCl, to a pH that was approximately the same as the pH of the methotrexate sodium reference solution. Using 0.2M HCl, the pH of the buffered solution was lowered to pH 6.55 and a spectral scan of the solution was obtained. Although some error was introduced

here in diluting the original solution with the volume of acid, the principal objective of this manipulation was to lower the pH of the buffered solution to a pH similar to the pH of the reference solution. This was done to determine whether the altered spectral occurring at the higher pH would revert back to the original spectrum. The resulting spectrum appeared not to change significantly from the altered scan, indicating possibly that, at the higher pH, an irreversible chemical change occurred. These results would tend to indicate that the change noted in the spectrum of methotrexate sodium, when it was combined with 5-fluorouracil in an admixture, was due to the change in pH of the methotrexate sodium. While not conclusive, it would also indicate that this chemical change is probably not reversible. These results are further substantiated by the results obtained by Hayden and co-workers (87) who observed different ultraviolet absorption spectra for methotrexate in 0.1N HCl (λ_{\max} 306 m μ) and in 0.1N NaOH (λ_{\max} 301 m μ).

It is of interest to note that, chemically, methotrexate represents an amphoteric substance in that it contains two basic amino groups and two acidic carboxyl groups. The chromophores may undergo change at different pH values and are most likely the cause in the changes in the spectrum of methotrexate sodium.

Although this method may have lacked accuracy, it did help to understand more completely, in one particular

case, the probable cause of the alteration of the spectrum of methotrexate sodium when in combination with 5-fluorouracil. Considering briefly the alteration in the λ_{\max} that occurred in the spectrum of 5-fluorouracil, it is of interest to note that the 5-halogenuracils, in general, have been reported to show a bathochromic effect, that is, a decrease or shift in the λ_{\max} of the ultraviolet absorption spectrum in response to pH changes. 5-Fluorouracil has been shown to demonstrate only a slight bathochromic shift (88). The significance of this, in respect to the effect this might have on its therapeutic efficacy, is uncertain.

The variables and limitations of absorption spectrophotometry in detecting chemical incompatibilities of intravenous admixtures has been reviewed by Catania (62). In this study, the infusion fluid used was 5 percent Dextrose Injection. While this vehicle is the one most widely used in medicine for the purposes of delivering medications, other I.V. fluids may be used. With others, it is entirely possible that the results obtained from this study might be different.

Limitations within the spectrophotometer itself could have had an influence on the results. It was noted that with sodium cephalothin, a certain amount of uncontrollable "noise" was obtained when scanning its spectrum. This "noise" was noticed in the region of λ_{\max} of the spectrum and, therefore, might be a concentration dependent problem with this particular agent.

While every attempt was made to use therapeutic concentrations of the drugs in the admixtures, in a few cases, for scanning, this was not possible because of the instrumental limitations. This difficulty necessitated dilution of the mixtures for evaluation.

CHAPTER V

SUMMARY AND CONCLUSION

Through the use of absorption spectroscopy and visual observations, the compatibility of selected oncolytic, antibiotic, and corticosteroid drugs was determined. The six drugs used in this study included methotrexate sodium, prednisolone sodium phosphate, sodium cephalothin, 5-fluorouracil, cytarabine, and vincristine sulfate. These were cross-matched in pairs, utilizing 5 percent Dextrose Injection, as the vehicle. By obtaining the ultraviolet absorption spectrum of each of the drugs alone in the 5 percent Dextrose Injection, reference or standard spectra were obtained which could be used as a comparison for the spectra of the drugs in admixture. This comparison permitted detection of any alterations in the spectrum from the admixture which might have been manifested by a loss of absorbance of a major peak in the spectrum, the emergence or disappearance of a peak, or in the general alteration of the spectrum.

The primary purpose of this paper was to determine any chemical incompatibilities that might result from chemical interactions of the drugs in admixture. Four possible chemical incompatibilities were noted, with one of the admixtures yielding significant alterations in the

absorption spectrum of each of the additives. None of the four possible chemical incompatibilities displayed any signs of a visual or "physical" incompatibility.

In conclusion, it can be said that spectrophotometric analysis does provide a useful tool for the determination of incompatibilities of intravenous admixtures. The results obtained here, however, can only serve as a guide to the clinician who wishes to mix drugs in the same infusion fluid. Further work in this area is still definitely needed and other methods of study need to be found. While probably the ultimate test of a drug's integrity and therapeutic efficacy still lie in biological testing, these methods have not yet been adequately developed and severe limitations are placed on time. Microbiological testing of the potency of a drug becomes greatly complicated and many other variables must be considered when done in the presence of other drugs. It is in many cases inappropriate.

It is hoped, as has been advocated by Donn (40), that the future of intravenous therapy will see a decrease in the number of I.V. admixtures used, and that attempts will be made not to admix drugs physically.

BIBLIOGRAPHY

1. Avis, K. E. Parenteral Preparations, "Remington's Pharmaceutical Sciences," 14th ed., Mack Publishing Company, Easton, Pa., 1970, p. 1519.
2. Dudrick, S. J. Rational Intravenous Therapy, Am. J. Hosp. Pharm., 28:82-92(Feb.), 1971.
3. Annan, G. L. An Exhibition of Books on the Growth of Our Knowledge of Blood Transfusion, Bull. N. Y. Acad. Med., 15:622-632, 1939.
4. Lancet Leader (1829). Transfusion: Lancet 1828-9, 2, 284, cited by Kerr, D. N.: The History of Intravenous Therapy, J. of Hosp. Pharm., 29:276-282(Oct.), 1971.
5. Latta, T. (1832) The Treatment of Cholera by Copious Injection of Aqueous and Saline Fluids Into the Veins. Lancet 1832-3, 1, 274, cited by Kerr, D. N.: The History of Intravenous Therapy, J. of Hosp. Pharm., 29:276-282(Oct.), 1971.
6. Elkington, J. R., and Danowski, T. S. "The Body Fluids," The Williams and Wilkins Company, Baltimore, Md., 1955, p. 8.
7. Parker, E. A. Parenteral Incompatibilities, Hosp. Pharm., 4:14-22(Aug.), 1969.
8. Superstein, E. The Need for Better I.V. Additive Studies, Drug Intelligence, 5:5(Jan.), 1971.
9. Parker, E. A. Solution Additive Chemical Incompatibility Study, Am. J. Hosp. Pharm., 24:424-439 (Aug.), 1967.
10. Latiolais, C. J. Implications of I.V. Additive Programs to the Parenteral Industry, Bull. Parenteral Drug Assoc., 24:45-51(Jan.-Feb.), 1970.
11. Meyers, E. L. Extemporaneous Mixing of Parenteral Medication, E.D.A. Papers, (June), 1967, pp. 14-16.
12. Kirkland, W. D., Jones, R. W., Ellis, J. R., and Schultz, C. G. Compatibility Studies of Parenteral Admixtures, Am. J. Hosp. Pharm., 18:694-699(Dec.), 1961.

13. Webb, J. W. A pH Pattern for I.V. Additives, Am. J. Hosp. Pharm., 26:31-36(Jan.), 1969.
14. Meyers, E. L. Extemporaneous Mixing of Injectables by Physicians and Pharmacists, Bull. Parenteral Drug Assoc., 19:164-171(Nov.-Dec.), 1965.
15. Carlin, H. S., and Perkins, A. J. Predicting Pharmaceutical Incompatibilities of Parenteral Medications, Am. J. Hosp. Pharm., 25:271-279(June), 1968.
16. Dunworth, R. D., and Kenna, F. R. Preliminary Report: Incompatibility of Combinations of Medications in Intravenous Solutions, Am. J. Hosp. Pharm. 22:190-191 (Apr.), 1965.
17. Misgen, R. Compatibilities and Incompatibilities of Some Intravenous Solution Admixtures, Am. J. Hosp. Pharm., 22:92-94(Feb.), 1965.
18. Plein, E. M. Preparation and Use of Intravenous Solutions: A Review of Incompatibilities and Related Problems, Hosp. Pharm., 4:5-13(Aug.), 1969.
19. Wagner, J. G. "Biopharmaceutics and Relevant Pharmacokinetics," First Edition, Drug Intelligence Publications, Hamilton, Ill., 1970, pp. 166-180.
20. Anon. Diphenylhydantoin Overdosage, Clin-Alert, No. 287 (Dec. 31), 1968.
21. _____. "Accreditation Manual for Hospitals," Joint Commission on Accreditation of Hospitals, 645 North Michigan Ave., Chicago, Ill.
22. Anon. Adding Drugs to Intravenous Solutions (Editorial) Lancet, 2:556(Sept. 12), 1970.
23. Peritore, S. P., and Rosenberg, J. M. I.V. Additives Move to the Pharmacy, Hospitals, 45:84-91(Dec. 16), 1971.
24. Skolaut, M. W. Educational Aspects of Intravenous Additives, Hosp. Pharm., 4:26-29(June), 1969.
25. Meyers, E. L. Extemporaneous Mixing of Injectables by Physicians and Pharmacists, Bull. Parenteral Drug Admixtures, 19:164-171(Nov.-Dec.), 1965.
26. Holysko, M. N., and Ravin, R. L. A Pharmacy Centralized Intravenous Additive Service, Am. J. Hosp. Pharm., 22: 266-271(May), 1965.

27. Francke, D. F. Preparation of Parenteral Admixtures--Nursing or Pharmacy Function? (Ed.), Am. J. Hosp. Pharm., 22:179(Apr.), 1965.
28. Meisler, J. M., and Skolaut, M. W. Extemporaneous Sterile Compounding of Intravenous Additives, Am. J. Hosp. Pharm., 23:557-563(Oct.), 1966.
29. Patterson, T. R., and Nordstrom, K. A. An Analysis of Intravenous Additive Procedures on Nursing Units, Am. J. Hosp. Pharm., 25:134-137(Mar.), 1968.
30. Pang, F. J. A Transition to Centralized I. V. Additive Service in a Private Hospital, Hosp. Pharm., 5:18-20 (Nov.), 1970.
31. Reifman, K. S. An I.V. Admixture Program in a Small Hospital: From Concept to Full Operation, Hosp. Pharm., 6:12-15(July), 1971.
32. Petruconis, S. K., and Newman, M. S. I.V.'s and Additives: Testing an Experience, unpublished study at the U.S.P.H.S. Hospital, San Francisco, Calif.
33. Goodwin, H. Developing a Parenteral Admixture Service in a Teaching Hospital (Columbus, Ohio: Ohio State University, 1966) through Peritore, S. P., and Rosenberg, J. M. I.V. Additives Move the Pharmacy, Hospitals, 45: 84-91(Dec. 16), 1971.
34. Carlin, H. S., and Perkins, A. J. Predicting Pharmaceutical Incompatibilities of Parenteral Medication, Am. J. Hosp. Pharm., 25:271-279(June), 1968.
35. Jacobs, J., and Superstine, E. Adding Drugs to Intravenous Solutions (Letters), Lancet, 2:1129(Nov. 28), 1970.
36. Francke, G. N., and St. Clair, H. I.V. Additive Services at Cincinnati Veterans Administration Hospital, Drug Intelligence, 5:377-380(Nov.), 1971.
37. Catania, H. Unpublished Survey taken at St. Joseph's Hospital, Stockton, Calif., 1972.
38. Miller, W. A., Smith, G. L., and Latiolais, C. J. A Comparative Evaluation of Compounding Costs and Contamination Rates of Intravenous Admixture Systems, Drug Intelligence, 5:51-60(Feb.), 1971.
39. _____. "I.V. Additives: Steps to Safety," Abbott Laboratories, North Chicago, Ill.

40. Donn, R. Intravenous Admixture Incompatibility Information Program, Bull. Parenteral Drug Assoc., 24:98-103(Mar.-Apr.), 1970.
41. Jones, R. W., Stanko, G. L., and Gross, H. M. Pharmaceutical Compatibilities of Pentothal and Nembutal, Am. J. Hosp. Pharm., 18:700(Dec.), 1961.
42. Anderson, R. W., and Latiolais, C. J. Physico-Chemical Incompatibilities of Parenteral Admixtures Aramine and Solu-Cortef, Am. J. Hosp. Pharm., 27:540-547(July), 1970.
43. Meyers, E. L. Extemporaneous Mixing of Parenteral Medication, F.D.A. Papers, (June), 1967, pp. 14-16.
44. Anon. "Selected References on Intravenous Additives," Office of Consultation on Hospital Functions, Health Care Facilities Service, Health Services and Mental Health Administration, Public Health Service, U. S. Dept. of H.E.W., Rockville, Md., pp. 1-11.
45. Bogash, R. C. Compatibilities and Incompatibilities of Some Parenteral Medication, Bull. Am. Soc. Hosp. Pharm., 12:445-448(July-Aug.), 1955.
46. Kirkland, W. D., Jones, R. W., Ellis, J. R., and Schultz, C. G. Compatibility Studies of Parenteral Admixtures, Am. J. Hosp. Pharm., 18:694-699(Dec.), 1961.
47. Riffkin, C. Incompatibilities of Manufactured Parenteral Products, Am. J. Hosp. Pharm., 20:19-22 (Jan.), 1963.
48. Im, S., and Latiolais, C. J. Physico-Chemical Incompatibilities of Parenteral Admixtures--Penicillin & Tetracyclines, Am. J. Hosp. Pharm., 23:333-343(July), 1966.
49. Patel, J. A., and Phillips, G. L. A Guide to Physical Compatibility of Intravenous Drug Admixtures, Am. J. Hosp. Pharm., 23:409-411(Aug.), 1966.
50. Gallelli, J. F. Stability Studies of Drugs Used in Intravenous Solutions, Am. J. Hosp. Pharm., 24:425-433(Aug.), 1967.
51. Parker, E. A. Solution Additive Chemical Incompatibility Study, Am. J. Hosp. Pharm., 24:434-439(Aug.), 1967.

52. Edward, M. pH--An Important Factor in the Compatibility of Additives in Intravenous Therapy, Am. J. Hosp. Pharm., 24:440-449(Aug.), 1967.
53. Webb, J. W. A pH Pattern for I.V. Additives, Am. J. Hosp. Pharm., 26:31-36(Jan.), 1969.
54. Ho, N. F. H., and Goeman, R. Prediction of Pharmaceutical Stability of Parenteral Solutions, Drug Intelligence, 4:69-72(Mar.), 1970.
55. Gill, A., Severson, R., and Ho, N. F. H. Prediction of Pharmaceutical Stability of Parenteral Solutions II, Drug Intelligence, 4:243-249(Sept.), 1970.
56. Ho, N. F. H. I.V. Additive Review Prediction of Stability of Parenteral Solutions III, Drug Intelligence, 5:47-51 (Feb.), 1971.
57. Simberkoff, M. S., Thomas, L., McGregor, D., Shenkein, I., and Levine, B. B. Inactivation of Penicillins by Carbohydrate Solutions at Alkaline pH, New Eng. J. Med., 283:166-199(July 16), 1970.
58. Anderson, R. W., and Latiolais, C. J. Physico-Chemical Incompatibilities of Parenteral Admixtures--Aramine and Solu-Cortef, Am. J. Hosp. Pharm., 27:540-547(July), 1970.
59. Zost, E. D., and Yanchick, V. A. Compatibility and Stability of Disodium Carbenicillin in Combination with Other Drugs and Large Volume Parenteral Solutions, Am. J. Hosp. Pharm., 29:135-140(Feb.), 1972.
60. Jacobs, J., Nathan, I., Superstine, E., and Sacks, T. Ampicillin and Carbenicillin Stability in Commonly Used Infusion Solutions, Drug Intelligence, 4:204-208(Aug.), 1970.
61. Riley, B. B. Incompatibilities in Intravenous Solutions, J. Hosp. Pharm., 28:228-240(Aug.), 1970.
62. Catania, P. N., and King, J. C. The Physico-Chemical Incompatibilities of Selected Cardiovascular and Psychotherapeutic Agents With Sodium Ethacrynate, Am. J. Hosp. Pharm., 29:141-146(Feb.), 1972.
63. Parker, E. A. Compatibility Digest, Am. J. Hosp. Pharm., 126:543-544, 412-414, 1969; 127:67-69, 327-329, 672-673, 1970.
64. King, J. C. "Guide to Parenteral Admixtures," Cutter Laboratories, Inc., St. Louis, Mo., 1970.

65. Meyers, F. H., Jawetz, E., and Goldfien, A. "Review of Medical Pharmacology," 2nd. Edition, Lange Medical Publications, Los Altos, Calif., 1970, p. 427.
66. Boesen, E., and David, W. "Cytotoxic Drugs in the Treatment of Cancer," Edward Arnold Ltd., London, Eng., 1969, pp. v-vi.
67. Ibid., p. 23.
68. Anon. "AMA Drug Evaluations," First Edition, American Medical Association, Chicago, Ill., 1971, p. 62.
69. Ibid., pp. 621-625.
70. Christian, D. G. National Cancer Institute, Baltimore Cancer Research Center, Baltimore, Md., Personal Communication, 1972.
71. Boesen, op. cit., p. 181.
72. Boesen, op. cit., p. 179.
73. Grimes, C. R. The Clinical Center, National Institutes of Health, Bethesda, Md., Personal Communication, 1972.
74. Anon. "Pharmacy Department Intravenous Additive Incompatibilities," Clinical Center, National Institutes of Health, Bethesda, Md. (Jan.), 1970.
75. Schou, S. A. Stability and Stabilization of Medicaments, Am. J. Hosp. Pharm., 17:5-10(Jan.), 1960.
76. Chakrabarti, S. G., and Bernstein, I. A. A Simplified Fluorometric Method for Determination of Plasma Methotrexate, Clin. Chem., 15:1157-1161, 1969.
77. Hunt, D. E., and Pittillo, R. F. Determination of Certain Antitumor Agents in Mouse Blood by Microbiological Assay, Cancer Res., 28:1095-1105(June), 1968.
78. Smolyanskaya, A. Z., and Agadzhanova, N. N. A Method of Determination of Concentration of Antagonists to Folic Acid in Blood and Urine in Chemotherapy, Vol. Onkol., 13:58-61(Sept.), 1967, through Chemotherapy, Chemical Abstracts, 67:11369g, 1968.
79. Udenfriend, S., Duggan, D. E., Vasta, B. M., and Brodie, B. B. A Spectrophotofluorometric Study of Organic Compounds of Pharmacological Interest, J. Pharmacol. and Exp. Therap., 120:26-32(May), 1957.

80. Gallelli, J. F., and Yokoyama, F. Assay of Methotrexate in the Presence of Its Decomposition Products and Other Folic Acid Analogs, J. Pharm. Sci., 56:387-389(Mar.), 1967.
81. Balazs, M. K., Anderson, C. A., and Lim, P. Rapid Assay Method for the Determination of Methotrexate, J. Pharm. Sci., 57:2002-2003(Nov.), 1968.
82. Anon. "The Pharmacopeia of the United States of America," 18th Revision, U. S. Pharmacopeia, Bethesda, Md., 1970, pp. 418-419.
83. Ascione, P. Application of Thin-Layer Chromatographic Techniques in Pharmaceutical Analysis, Bull. Parenteral Drug Assoc., 23:137-141(May-June), 1969.
84. Johnson, J. B., and Venturella, V. S. Specificity of Analytical Techniques in Evaluating Stability of Parenterals, Bull. Parenteral Drug Assoc., 25:239-246 (Sept.-Oct.), 1971.
85. Trujillo, A. P. "Thin-Layer Chromatography of the Platinum Metal Ions," Unpublished Thesis in partial fulfillment of Doctor of Philosophy degree at the University of the Pacific, Stockton, Calif. (Aug.), 1965.
86. Anon. "Remington's Pharmaceutical Sciences," 14th Ed., Mack Publishing Company, Easton, Pa., 1970, p. 284.
87. Hayden, A. L., Samuel, O. R., Selzer, G. B., and Carol, J. Infrared, Ultraviolet, and Visible Absorption Spectra of Some USP and NF Reference Standards and Their Derivatives, J. Assoc. Official Agricultural Chemists, 45:840(Nov.), 1962.
88. Wempen, I., and Fox, J. J. Spectrophotometric Studies of Nucleic Acid Derivatives and Related Compounds, VI On the Structure of Certain 5- and 6-Halogenuracils and-cytosines, Am. Chem. Soc. J., 86:2474-2478(June), 1964.